SEED BORNE DISEASE OF BENGAL GRAM

(Cicer arietinum)

A Thesis
Submitted for the Degree of
Doctor of Philosophy

in
Science
to the

University of Allahabad

By

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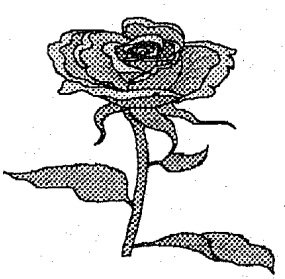
Dedicated

to

My Beloved

Parents



CERTIFICATE

Certified that the thesis embodies results of original research work and study carried out under my supervision by Mr. Sudhir Kumar Tiwari M.Sc. (Ag.) Botany.

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PREFACE

The thesis embodies research work on **"Seed-borne disease of Bengal Gram (*Cicer arietinum*)"**. The work carried out by me from December 1996 to March 2002 in the Bhargva Agricultural Laboratory, Garden and Farm of Botany Department, University of Allahabad.

The thesis embodies researches with studies on some seed-mycoflora isolated from Bengal gram seeds. The thesis contains work on Isolation and Pathogological studies, Effect of Storage Conditions, Bio-chemical Studies, Studies of some isolated mycoflora with their respective host seeds and control studies.

The thesis is organised into Ten Chapters. First chapter deals with Introduction followed by Materials and Methods (Chapter - 2) Isolation and Pathological Studies are discussed in Chapter - 3. Storage Studies and Bio-chemical Studies are given in Chapter - 4 and Chapter - 5 respectively. Chapter - 6 is Survival Studies. Control Studies are discussed in Chapter-7.

A detailed Discussion on the basis of the present investigation and the Summary are given in Chapter - 8 and Chapter - 9. The bibliography containing the references cited in the text is appended in the end (Chapter - 10). Abstract of the present work is also submitted along with the thesis separately.

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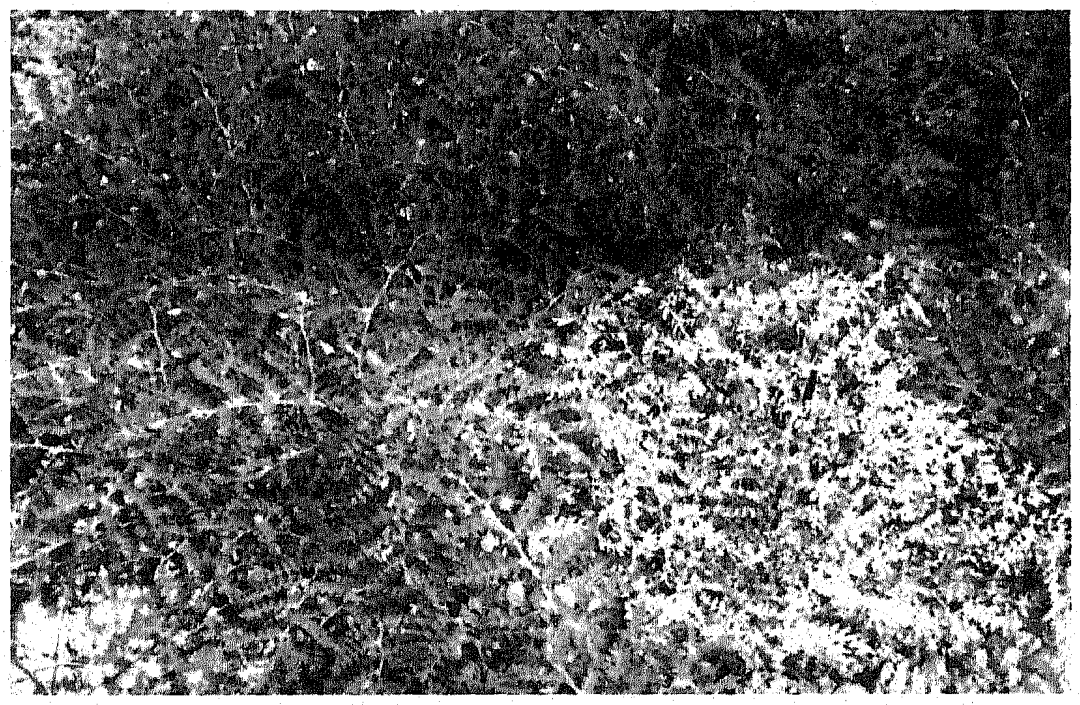
CONTENTS

CHAPTER -1

Introduction



INTRODUCTION

Pulses are an integral part of the vegetarian diet in the Indian sub-continent. Besides being a rich source of protein they maintain soil fertility through biological nitrogen fixation by bacteria prevalent in their root nodules and thus play a vital role in furthering sustainable agriculture. Since time immemorial, pulses have been cultivated in rainfed condition, which are characterised by poor soil fertility and moisture stress (poor water retentive soils). These crops are energy rich but are cultivated largely under energy starvation conditions. More than 78 percent of the area under pulses is still rainfed, and therefore, productivity has not increased as it should have after the release of dozens of improved varieties .

It is estimated that our country's populationlation will touch nearly 1350 million by 2020 A.D. The country would then need a minimum of 303 million tonnes of pulses.

The total area under pulses remained virtually stagnant (22-24 million hectares) with almost stable production (12-14 million tonnes) over the last four decades. The area increased in Madhya Pradesh, Andhra Pradesh, Karnataka, Maharashtra, Gujarat and Tamil Nadu while it is almost stagnant in Rajasthan. It has decreased in Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal. This decrease in area may be due to increase in irrigation facilities in these states resulting in shift from pulses to cereals.

Individual crops have undergone significant changes with respect to area, production and productivity. The area and productivity of chick pea has drastically come down in Bihar, U.P., Punjab, Haryana and Rajasthan in northern India while it has gone up in Madhya Pradesh, Karnataka, Maharashtra and Gujarat. Productivity over the years has shown an increasing trend in Maharashtra, Gujarat and Rajasthan.

The per capita availability of pulses has declined from 64 g/capita/day (1951-56) to less than 40 g/capita/day as against the FAO/WHO's recommendation of 80 g/capita/day. If we take into account the total protein nutrition derived from other protein sources such as foodgrain, milk and its products, eggs, fish, meat, etc. then 55 g/capita/day requirement of pulses may be the realistic target. (The Hindu survey of Indian Agri. 1999).

The main qualities of pulses are provide two to three times more protein than many cereals and its protein composition makes up for the deficiency of essential aminoacides in cereals and millets. Pulses fix atmospheric nitrogen in their roots and thus improve soil fertility. The tap root system opens the soil into deeper strata and heavy leaf drop increases the soil organic matter.

Bengal Gram (*Cicer arietinum* L.),is commonly known as "Chick pea", Bengal gram is an important grain legume in the India sub-continent, West Asia, Northern and Eastern Africa and Central and South America. It is known by other names such as gram. Spanish pea, Chestnut bean (English), Pais chiches (French) Homos (Arabic). Garbanzo (Spanish) and Chana (Hindi). According to the I998/FAO production year book.

Bengal gram occupies a position of pride among the Leguminous crop owening to its great importance both as vegetable and as pulse. In a country like India where most of the population is primarily vegetarian Bengal gram has a special place in the daily diet of people due to its high protein content and manifold uses. A large population in this country is already suffering from protein malnutrition due to short supply and high prices of pulses which have gone beyond the reach of poor man. Therefore, if the over increasing population is to be fed properly in boost up the production of this important pulse and vegetable crop.

Bengal gram belongs to the sub-family *papilionaceae* of family leguminaceae. Its probable place of origin lies in South Western Asia, that is, in the countries lying to the North-West of India such as Afghanistan and Persia. According to Aykroid Doughty (1964), the centre of origin of gram is Eastern Mediterranean. The fact that Bengal Gram has a Sanskrit name which indicated that the crop has been under cultivation in India longer than in any other country (Decandalle, 1886). It thrives in the cool climate, therefore it is grown mainly during winter season but severe cold frost are injurious to it. It is generally grown under rainfed conditions but is given good returns in irrigated conditions as well. It is best suited to areas having moderate rain-fall of 60-90 cm per annum.

Among the various grain legumes, dry Bengal gram is of worldwide importance and stands first both in production and acreage of pulses in the world. In India, Bengal gram is grown in almost all the states both for any grain and green pod. It is cultivated on 7.22 million hectares with annual production of 6.01 million tones and average yield 832 Kg/h.

The Bengal gram is consumed in different forms. Dry Bengal gram are used as split Bengal gram (Dal) and Besan for various salty and sweet preparations. Both husks and bite of dal are valuable cattle feed. Fresh green leaves are used as vegetable (Sag). Straw of gram is an excellent fodder for cattle. The grains are also used as vegetable (Chhole). It is the leading vegetable among frozen foods. Gram is considered to have medicinal effects and it is used for blood purification.

Bengal gram surpasses most of the vegetable and other crops in nutritive value. It contains 21.1% protein, 61.5% carbohydrate, 4.5% fat. It is also rich in calcium, iron and niacin.

Inspite of the evolution of improved varieties and adoption of recommended package of practices, the average production of this crop is very low in India in comparison to many other countries of the world. Among the various factors, responsible for lowering down its yield, the diseases especially these caused by fungi are considered to be the major ones. For enhancing the production of this important pulse and vegetable crop in order to keep pace with the mammoth increase in population of this country, it is imperative to make every effort for combating the maladies which take away a heavy tall of the crop every year.

Bengal gram (*Cicer arietinum* L.), being an important pulse vegetable crop, has attracted the attention of plant pathologists all over the world, where ever this crop is grown. A critical review of literature has revealed that Bengal gram suffers from a number of diseases caused by fungi in addition to bacteria, viruses and nematodes which lower the quantity and quality of the product of this crop. Some of the common

diseases and their causal organisms reported on this crop are given below :

Major disease of Bengal gram alongwith their causal organisms reported from India. Floral blight of gram *Alternaria alternata* (Vishwakarma and Basu Chaudhary 1974).

Floral blight of gram, *Alternaria alternata*, Vishusakarma and Basu Chaudhary (1974). Alternaria blight, (*Alternaria circinans)*, Guraha at al., (1981), Seed and root rot, *Alternaria* state of *pleospora infectoria*, Haware and Seene (1976), Alternaria blight, (*Alternaria tenuissima)*, Narain, Yadav and Sinha (1990), Ascochyta blight, (*Ascochyta mycosphaerella)*, Aga (1966), Botrytis gray mold, (*Botrytis cinerea),* Joshi and Singh (1969), Leaf spot of gram, (*Colletotrichum dematium)*, Mishra, Sharma and Joshi (1975), Fusarium wilt of gram, (*Fusarium solani),* Grevel, Pal and Kulshreshtha (1974), Wilt of gram, (*Fusarium orthocerae)*, Anonymous (1947), Foot rot, (O*perculell padwickii),* Among mous (1974), Blight of gram, (*Phyllosticta rabiei)*, Aujla (1960), Seed rotting, (*Pythium ultimum)*, Anonymous (1954), Dry root rot, (*Rhizoctonia beticola)*, Dastur (1935), Stem rot, (*Sclerotinia sclerotiorum)*, Bedi (1956), Collar rot, (*Sclerotium rolfsii),* Anonymous (1947) Mathur and Chauhan (1968), Stemphylium leaf spot, (*Stemphylium sarcini forme)*, Das and Sen Gupta (1961), Rust, (*Uromyce ciceries arietani)*, Asthana (1957), Rot of Chickpea, (*Xanthomonas cassiae)*, Rangaswami and Prasad (1960), Chickpea stunt, (*Chickpea stunt viruses)* transmitted through Aphids craccivaria, Nene and Reedy (1976), Phyllody, (*Mycoplasma transmitted),* through Vasudeva

and Sahambi (1957), Root knot, (*Meloidogyne incognita),* Ahamad Jamal (1976).

About 50 pathogens have so far been reported on Bengal gram from different parts of the world (Nene, 1979) while some reports are mere records of their occurrence, many diseases are wide spread and few are devastating. A survey of literature reveals that only a few diseases have been investigated in detail. (Nene, 1979).

Of the several disease recorded on Bengal gram very few are reported as seed borne. The seed borne nature of *Ascochyta rabiei* was described by Luthra and Bedi (1932) and subsequently confirmed by madeneral (1975). Haware et al. (1978) described the seed borne nature of F. oxysporum, F. sp. ciceri. The possibility of seed borne nature of the powdery mildew (*Oudiopsis taurica)* of Bengal gram was ruled out by studies carried out by Haware and Nene (1979).

The disease can be observed in susceptible cultivar within 25 days after sowing in the field. Seedlings that die due to the wilt disease can be confused with those that die due to root rot if not examined carefully.

The affected seedlings show dropping of the leaves and paler colour than the healthy seedlings. They may collapse and lie flat on the ground such seedlings when removed from soil show shrinkage of the stem. The roots do not show shrinkage of the stem the roots do not show any external rooting but look apparently healthy. Such roots when split vertically from the collar region downward show a brown discoloration of the internal tissues.

Adult plants show typical wilting, which may occur in the field upto podding stage. The initial symptom is dropping of petioles and rachis along with leaflet. Within 2 to 3 days dropping is seen on the entire plant. There is a slow fadingof the green colour and the plant looks dull green. Gradually all leaves turn yellow and straw coloured roots of the wilted plant show no external rotting drying or discoloration (Nene et al. 1979) Root and stem of the wilted plant when split vertically clearly show internal discoloration of the pith and xylem.

Two types of Bengal gram are cultivated small seeded desi type with generally brown to bright yellow testa colour and large seeded kabuli type with salson white testa colour large seeded types are the characteristic of the Mediterranean and western hemisphere areas of production and small seeded types of the Indian sub-continent. White or light coloured large seeds compared the highest price in Indian market. The annual production of Bengal gram in India is too low as compared to its average. The yield of Bengal gram besides other factors, is greatly affected by diseases like wilt, root rot, stunt, Grey mould *Ascochyta blight* rust and Athens. Wilt diseases alone are empirically estimated to cause about 10% looses in yield which accounts approximately 601 thousand tonnes annually in the country and in turn results in an approximate loss of national income about Rs. 16,74,400/-.

If the losses caused by wilt disease alone could be investigated country will be annually benefited, substantially. This is only possible when an economic control measure of this disease becomes available to the farmers. The most economic control measure can be the availability of resistant varieties. This is only possible when an uptodate knowledge

about the physiologic specialization resistance of races of the pathogen, distribution of these races in different regions of the country and the knowledge of various *Fusarium species* involved in the wilt complex of Bengal gram investigated.

The wilt of Bengal gram is relatively more serious than other known disease and has been reported from Burma, India, Mexico, Pakistan, Peru and the U.S.A. (Nene, 1978).

In Northern India wilt is often referred to as early or late wilt, depending upon the line of occurrence. Early wilt refers to seedling wilt (October-November) and late wilt refers to wilting at post flouring stage (February-March). Generally the wilt incidence is negligible in the intervening period in some part of India, wilt in Bengal gram occurs at any time from the seedling to the padding stages. First mention of Bengal gram wilt in India dates back to early part of this country Butler (1918) and Mckernal (1923), from Eurma indicated the nature of this disease to be soil borne and specimens yielded *Fusarium* sp. Nersimhan (1929), reported the association of *Fusarium* sp. and *Rhizoctonia* sp. with Bengal gram wilt disease. Later, Dastur (1935) Found *Rhizoctonia beticola* causing wilt in Bengal gram and he designated this as 'Rhizoctonia wilt' Even though he isolated *Fusarium* sp. from several wilted plants, but failed to reproduce the disease artificially. However, the description of the symptoms and pattern of incidence in field was more or less identical to those or wilt typically used by *Fusarium oxysporum F. sp. ciceri*. Any how he concluded the wilting due to physiological reasons and assigned it as '*Physiological wilt*'. Prasad and Padwick (1939), reported *Fusarium* sp. to

be the cause of chickpea wilt. The Fungus was letter named by Padwick (1940), as *F. orthoceras* var *ciceri*. Fruein (1958), from the U.S.A. reported *F. lateritium F. sp. ciceri* to be the causal organism of wilt and questioned the earlier nomenclature as F. orthoceras vas *ciceri*. Following the classification of Shyder and Bansen (1940).

Chattopadhyay and Sen Gupta (1967) renamed *F. orthoaras Var. Ciceri as F. oxysporum, F. sp. Ciceri.* This change has been accepted by Booth (1971). Since then *F. oxysporum F. sp. Ciceri* is uniformly accepted universally as the caused agent of wilt in Chickpea.

Ascochyta blight is one of the most important disease of chickpea. Particularly in Pakistan West Asia and Northern Africa during 1980-81 and 1981-82 seasons, Ascochyta blight appeared in epiphytic from in part of Punjab and epiphytic form n part of Punjab and Haryana state of India. Nene (1982)has reviwed the Ascochyta blight.

Initial symptoms on the seedlings are in the form of elongated, sunken, dark lesions.on the stem near the soil surface. The symptoms may appear because of seed borne inoculum or because of the presence of Fungus in the soil. Symptoms at the base of the stem remain restricted in the absence of high humidity and cool temperature.The plant may then grow normal, though a little weak fungus in the seed starts sporulating before the seed germinates and masses of Rhchidiospores may come in contact with the emerging shoot resulting in the injection at the stem base.

Out breaks of the disease in fields are associated with cooler day temperatures (20-25°C) extended cloudy days and intermittent rains accompanied by winds.

Field symptoms are well documented in the literature (Luthra and Bedi, 1932) All above ground parts are attacked because of favorable microclimate around the stems and lower branches brown to dark brown elongated lesions appear on the stem. These lesions girdle the stems or the base of the branches and the plant part above the lesions rapidly die on the leaflets the lesions are initially round and water soaked. Later they develop into well defined rained or elongated brown to dark brown spots with Sunken tissues and dark margins with nakedly pycnidia(as dark black bodies) can be observed partly embedded in host tissues, in concentric circles. As the disease advances in the field patches of diseased plants suddenly become prominent and may increase future. The lesions on the pads are round with dark margins and pychidia are distributed in concentric circles in Grey tissues. The developing seed is also infected.

Severally, infected seeds are small wrinkled and have dark brown lesions of various shapes and sizes. The lesions are more prominent on white seed. Also pycnidia can be observed in deep lesions on such seeds. If the pad infection occurs at maturity, a normal looking seed may show slight discoloration on the surface. Even the seed which looks apparently healthy may harbour the pathogen.

A grey mould was reported for the first time from Argentina (Carranza, 1965). It was then reported desing 1967-68 season from Pantnagar in India (Joshi and Singh, 1969) During 1980-81 and 1981-82 seasons. There was aschere out break of this disease along with Ascochyta blight in northern India. Only then the importance of grey mould disease was realized.

The disease appears on the flowers, leaves, branches and stems in the form of grey to brown lesions, covered with erect hairy sporophores and a mass of single celled hyaline spores. The fungal growth is evident on flowers and petioles if observed early in the morning drooping of the affected. Terminal tender branches is a common symplam in the field. In cloudy weather, flower drop and rotting of plant parts is quite conspicuous. The entire foliage becomes discoloured.

With low humidity irregular brown spots are observed on the leaves. The spots are circular to elongated on the stem some times tiny black scleratial masses can be observed on dead tissues. These sclerotia are small, dark bodies and should not be confused with larger black or dark brown sclerotia embedded in the white mycelial mat of *Sclerotinia sclerotiorum.*

Pods are also attacked. At that time, no seeds or only small, shriveled seeds are formed in the affected pods. Grayish white mycelium is seen growing on immature seeds. Lesions on the pod are water soaked, irregular and sometimes black sclerotial bodies scattered in the infected areas are observed.

Colletotrichum blight disease was reported from Madhya Pradesh in India (Mishra et al. 1975) Coletotrichum blight is observed in high humidity conditions with a 30°C day temperature. It is regularly observed during September-October in early sown Bengal gram crop with high day temperatures and intermittent rain. The disease has potential to become as serious as *Ascochyta* blight and *Botrytis* grey mould.

The disease may appear in the field and kill the Bengal gram plants at any stage of growth depending on the weather condition and inoculum.

On seedlings two kinds of symptoms may be observed (1) elongated sunken dark brown spots on the lower part of the stem extending to the root and (2) Wilting and drying of the seedling due to severe root infection.

On adult plant lesions are seen on the leaves stems and pods, lesions are circular to elongate sunken in the middle and surrounded by yellow margins on stem they are elongated and black on pods the acervuli along with black setae are scattered within the stromatic tissues. The fungus is able to penetrate the pod-wall and infect the seed.

Alternaria blight is sometime observed in Bengal gram. The disease, is seen mostly in the adult stage. Floral blight of Bengal gram caused by *Alternaria alternata* was reported from Varanasi. India (Vishwakarma and Chaudhary, 1974). However the pathogen attacks all aerial part (Haware and Nene, 1980) *Alternaria* state of *Pleospora infectoria* was also found to cause Bengal gram blight (Haware and Nene(1980).

All the part of Bengal gram plant are attacked. Infection in generally severe on leaves. Lesions on leaflets are water soaked initially, restricted in size circular and purple in colour. They are surrounded by chlorotic tissues but without definite margins. Lesions later turn brown to dark brown. With high humidity the lesions coalesce. Cover the leaf area and cause rapid withering of the individual leaflets sporulation can be observed on necrotic tissues under stereo binocular microscope.

One the stem the lesions are elongated and brown to black while on the pods these are circular slightly sunken and scattered irregularly. Pods become dirty black seed is infected and shriveled. On mature pods ting, black superficial flecks remain localised.

Two species of *Alternaria* have been reported to be associated with Bengal gram cause the foliar disease at Kanpur. They are leaf blight and venial necrosis caused by *Alternaria circinans* Guraha (1981) and leaf blight caused by *Alternaria tenuissina* Narain et al. (1990).

An *Alternaria* blight of Bengal gram caused by *Alternaria alternata* (Er.) Keisley was reported from Rajasthan, India Gaur and Singh (1990) A similar disease, *Alternaria pod* blight gram (*Cicer arietinum)* was observed by Rout and Somanic (1980) from Vidarbh Maharashtra. This is the first record of pod blight caused by *Alternaria alternata*.

During 1985-86 diseased Bengal gram plants and seed samples were investigated and *Alternaria alternata* was observed on all plant aerial parts, rot necks and seeds (Simay, 1989).

Among the maladies of Bengal gram, leaf blight caused by *Alternaria alternata* is much importance as it appears more severally and is much destructive ones on Bengal gram crop in Uttar Pradesh because most of the commercial and improved varieties were observed to be affected from the disease during the recent years.

Durinng the current study work was undertaken as an integral part of a comprehensive plan of experimental investigation to find out results for the furtherance of scientific knowledge. Efforts were made to find out

detailed studies regarding the severity of the problem in the local area. The investigations were conducted with recognized methodology. Attempts have been made to study the fungal flora. Some known and unknown varieties of Bangal gram. An attempt have been made to isolate fungi from seeds of Bengal gram stored at different length of time.

Fungi were isolated cultured, purified, maintained in different culture media and identified. Effect of moisture control present in different seeds of Bengal gram and percentage of seeds contaminated with fungal flora has also been observed. Pathogenicity test have been carried out with fungal flora isolated from seeds of Bengal gram. Symptoms if produced have been recorded on their respective host effect of fungicides have been evaluated in the laboratory against some pathogenic form and the successful one's have been further tried in the field conditions.

The present studies have enabled to generate information about the various *Fusarium* species involved in the wilt complex of Bengal gram in India. The distribution of these species in different agroclimatic regions has been investigated. Existence of races of *F. oxysporum, F. sp. ciceri* the main pathogen of Bengal gram wilt has been workout. The reaction of Bengal gram germplasm types to different *Fusarium* species individually has been determined. The performance of *Fusarium* species against various Bengal gram genotypes i.e. host pathogen interaction forms interesting aspects of knowledge. The information generated from the present studies are aspected to achieve the goal for economic gains and stable control measure in addition to find out resistant varieties.

CHAPTER - 2

Materials And Methods

MATERIALS AND METHODS

During the year 1997-98 a survey for prevalence and severity of seed borne diseases of Bengal gram was made in different agro-climatic conditions, such as Allahabad, Banda, Hamirpur, Mahoba, Mauranipur (Jhansi) Regional Agricultural Testing and Demonstration Centre Lahartara (Varanasi), I.I.P.R. Kalyanpur (Kanpur) and its adjacent regions was made. Various seed samples of Bengal gram or Chickpea crop was collected. Blatter and the agar plate methods use for detection of seed borne and surface fungi. An attempt was also made to isolate fungi from some split pulses.

Various seed samples of Bengal gram crop were collected from I.A.R.I. New Delhi, Allahabad Agricultural Institute, Allahabad, G.B. Pant University of Agriculture and Technology, Pantnagar and from local markets. Fungi were isolated, purified and maintained on Malt Extract and Potato Dextrose Agar media, for further examination and pathogenicity tests. Morphological studies were carryed out and identifications were made.

The seed were brought to laboratory and stored in sterilized glass containers to study the surface fungal flora seeds were directly placed on petridishes. Containing malt extract agar medium and on mist sterilized blotting paper.

100 seeds of Bengal gram crop were taken in each case. To study the internal seed fungal flora, the seed were dipped in 0.1% mercuric chloride for 2-3 min, rinsed several time in sterilized distilled water and were placed similarly as above on petridishes containing malt extract agar on moist sterilized blotting paper.

Petri dishes containing seeds were incubated for 7 to 10 days at 25°C ± 1°C. Any fungal growth observed on seeds during this period was picked up and subsequently grown on Malt Extract or P.D.A. on Asthana and Hawkers medium 'A' for further studies. The fresh stock of cultures was maintained by subculturing the fungus at regular intervals of three weeks on Potato Dextrose Agar medium and also where it was necessary single spore cultures of the fungi were prepared with the help of dummy cutter objective. The following media were used for isolation and other studies.

1. Malt Extract Agar Medium (Raper and Thom 1949).

Agar	25.0 gm
Malt extract (Difco)	20.0 gm
Dextrose	20.0 gm
Peptone	1.0 gm
Water (distilled)	1000.0 ml.

2. Potato Dextrose Agar medium (Riker and Riker, 1936)

Agar	17.0 gm
Potato	200.0 gm
Dextrose	20.0 gm
Water	1000.0 ml
pH	6.0 - 6.5

200 gm of potato was washed, cut into small pieces and boiled half an hour in 500 ml distilled water and then filtered through muslin cloth 20 gm of dextrose was added to it and final volume was made-up to 1000 ml.

3. Asthana and Hawker's medium 'A'

Glucose	5.0 gm
KNO_3	3.5 gm
Potassium dihydrogen Phosphate	
KH_2PO_4	1.75 gm
Magnesium sulphate ($MgSO_4$ $7H_2O$)	0.75 gm
Distilled Water	1000 ml.

To solidify 20 gm Agar agar was added.

The method used for taking out soil samples was similar to that used by Saxena and Mehrotra (1952) and Sarbhoy (1963). Samples were taken from all the four sides of the pit and at different depths (3", 6", 9" and 12") and the samples from all the depths of a locality were mixed together. They were packed in sterilized polythene bags and brought to the laboratory. Samples were air dried, crushed and passed through 2 mm sieve.

The soil plate method was employed to isolate the various *Fusarium* species as well as to count the number of *Fusarium* colonies in a particular sample. Modified Czapek Dox Agar medium (Singh and Nene, 1965) containing sodium nitrate 2.0 gm, dipotassium monohydrogen phosphate 1.0 g magnesium sulphate heptehydrate 0.5 gm, ferrous

sulphate 0.01 gm sucrose 30 gm agar agar 20 gm and distilled water to take 1000 ml was used. After autoclaving fresh solution of malachite green (50 mg/1), Captan (100 gm/1) and Dicryaticin (0.75 mg/1) were added. The medium was lightly cooled and 20 ml was poured into each plate to solidify. 50 mg of the soil was sprinkled on solidified surface of the medium and the petridishes were incubated at 25° ± 1°C for 3-5 days.

Disease part of some pulse crops roots were washed with water, cut into small bits, surface sterilized with 0.1 percent mercuric chloride solution and finally washed with several changes of sterilized water. The sterilized root bits were placed into sterilized Potato Dextrose Agar medium (Peeled potato 250 g, agar bag, dextrose 20 gm distilled water 1000 ml). Previously poured into sterilized petridishes by half plate method. The plates were incubated at room temperature (28°C ± 2°C) profuse growth of the mycelium was seen with in seven days.

The isolated fungus was artificially inoculated in pot soil through sand maize medium at 20 percent (W/W) when pulse seeding planted in these pots and field were 45 days old. The plants exhibited wilt symptoms in a period of 25 days. The pathogen was reisolated from these artificially inoculated plants and maintained on oats slants for further studies.

Preparation of Plant extracts :

Cold distilled water extraction : A 50gm of leaf was washed, chopped and crushed using mixie with 100 ml. of distilled water and filtered through muslin cloth. Then the filtrates were used for inhibition zone technique by paper disc method and poisoned food technique.

Hot distilled water extraction. In this method distilled water at 70°C was used instead of cold distilled water. Then these extracts were used for inhibition zone technique by paper disc method and poisoned food technique. The leaf material used for cold distilled water extraction alone was used for hot distilled water extraction at the same concentration.

Testing fungitoxicity of the plant extracts, the method followed by Eben and Keit (1950) was slightly modified and carried out. Sterlized filter paper discs (10mm) were soaked in cold and hot distilled water plant extracts and 50% concentration. In each of the petriplates two treated discs were placed at the centre on potato dextrose agar medium. Suitable controls were maintained by placing the discs soaked in sterile water. The mycelial discs (9 mm) were kept at three places on the periphery of petriplates at equal distance. Each treatment was replicated three times and incubated at room temperature (28 ± 2°C) for 24 hours.

The effect of plant extracts on the growth of the pathogen was studied by poisoned food technique Grower and Moores (1962). A 50gm of different plant leaf materials extracted in 100 ml. of hot and cold distilled water were used. Five ml. of these extracts were taken to incorporate into 50 ml. of potato dextrose agar medium and autoclaved for 20 minutes at 1.41 Kg/cm^2 pressure. The plant extract incorporated medium at 5% was poreed into the sterlized Petriplates and allowed to cool. The plate were inoculated with uniform discs of 9 mm diameter from 3-day old culture grown on potato dextrose agar medium the diameter of colony growth was recorded after 24 hours and the mean inhibition percentage worked out. The inoculated Petriplates were incubated at

room temperature (28 ± 2°C) for 24 hours with suitable control.

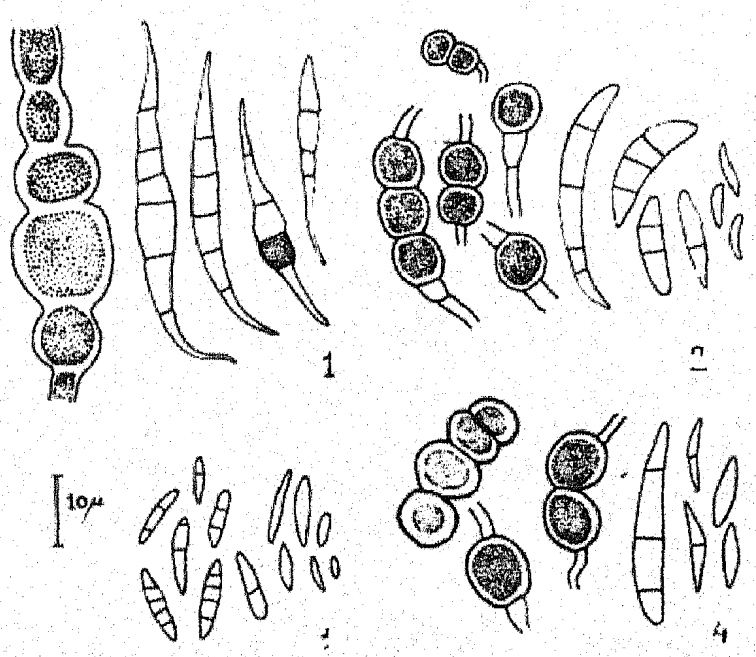
The treated and sterlized water soaked seeds were kept for germination in roll towel and each treatment was replicated three times. The seeds were kept for germination at 25 ± 5°C and 95 ± 2 percent relative humidity.

Plant extracts tested under roll towel method were applied on the pulses seeds at 50% concentration for 6 hours and then shade dried for 2 hours. Pathogen multiplied on sand maize medium (19 : 1 radio) was used for the inoculation of unsterilised soil. The innoculum was mixed at the rate of 5% to the soil and it was incorporated one day before sowing. Each treatment was replicated three times. One hundred seeds were used for each treatment and the observation were recorded. Similar experiment was also conducted to assess the efficacy of storage seed treatment on the seedling mortality.

Pathogen multiplied on sand maize medium was incorporated into the unsterilised soil one day before sowing at the rate of 5gm per 100g of soil in earthern pots. Pulses seeds were sown in inoculated soil and the plant extracts used for seed treatment were also used for soil drenching at 25% concentration immediately after sowing. Drenching the soil was given upto 5 cm depth. Seeds sown in pathogen inoculated and uninoculated control were also drenched with tap water. Each treatment was replicated three time. One hundred seeds were used for each treatment.

CHAPTER - 3

Pathological Studies



PATHOLOGICAL STUDIES

(1) ISOLATION

Mckernal (1923) for the first time reported that Bengal gram (*Cicer arietinum)* wilt in Burma is caused by a *Fusarium* sp. closely allied to the fungus *F. udum* attacking *Cajanus sp.* in India. Rhind (1926), isolated certain *Fusarium* species in addition to other fungi from wilted Bengal gram plants, However, inoculation experiments with these organism gave negative results. McRae (1929), opined that an un-determined species of *Fusarium* was responsible for wilt of Bengal gram in the districts of Coimbatore and Bellary in India. Pedwick (1939) in periodical isolations from wilted plants of Bengal gram obtained a number of *Fusarium* species. In further studies, Padwick (1940), sumarised that the fungi causing wilt in Bengal gram comprised only one variety. He further considered that the *Fusarium* causing Bengal gram wilt was either a new species or merely a physiologic race of *F. orthoceras var pisi* Anonymous (1947), reported that wilt developed in only those Bengal gram plants which were inoculated with were inoculated with *Fusarium orthoceras* alone. Anonymous (1952) found that of the various fungi concerned in the etiology of Bengal gram wilt, *Fusarium orthoceras* induced typical wilt symptoms in the inoculation experiments. Anonymous (1954) observed that in the late December sown Bengal gram plants wilted severally *Fusarium othocerae* var *ciceri*. Erwin (1958) from California reported that

the fungus isolated and that caused wilt in Bengal gram was *Fusarium* lateritian.

Chattopadhyay and Gupta (1967), on the basis of studies with the monocordial isolates from discussed chikpea proposed that *Fusarium orthoceras* F. sp. *ciceri* should be renamed as *Fusarium oxysporum* F. sp. *ciceri* Kalser et al. (1968) found an unidentified *Fusarium* sp. causing root rot in Bengal gram in Iran. Cother (1977),observed that *F. orthosporoides* and *F. avenaclum* were associated with Bengal gram wilt in New South Wales. Several sp. of *Fusarium* have been reported to be associated with Bengal gram. wilt in different countries in the world. There is a need to investigate thoroughly about the *Fusarium* species associated in Bengal gram wilt in India.

Sl.No.	**Name of the Disease**	**Causal Organism**
1.	Bengal gram wilt	*Fusarium oxysporum schlecht*
2.	Ascochyta Blight	*Ascochyta rabies (Pass)*
3.	Botrytis gray mould	*Botrytis cinerea*
4.	Calletotrichum blight (Leaf spot of gram)	*Colletotrichum dematiam*
5.	Floral blight of gram (Leaf blight of Bengal gram)	*Alternaria alternata*

Table : 1

Prevalence and severity of seed borne disease of Bengal gram at different locations in Uttar Pradesh.

S.No.	Location	Disease Intensity %
1.	Naini Agriculture Institute Farm Allahabad	32.00
2.	Crop Research Farm Banda	41.00
3.	Crop Research Farm, Mauranipur, Jhansi	33.00
4.	Crop Research Farm Gursarai, Jhansi	33.00
5.	Indian Institute of Pulse Research Farm, Kalyanpur, Kanpur	38.00
6.	Crop Research Farm, Mahoba	34.00
7.	Regional Agricultural Testing and Demonstration Centre, Varanasi	26.00
8.	Allahabad University Farm, Beli, Allahabad	25.00
9.	C.S. Azad University Legume Research Farm, Kanpur	36.00
10.	Bithoor, Kanpur	29.00

Therefore, with a view to assess the prevalence, severity and distribution of the disease, a survey was conducted during the crop season 1997-98 in different Bengal gram growing areas of Uttar Pradesh, representing important agroclimatic conditions. The data collected are presented in Table 1.

The results presented in Table 1 reveal that the disease was prevalent in all the areas surveyed showing its wide spread occurrence. The maximum disease intensity was recorded at the Crop Research Farm Banda followed by Indian Institute of Pulse Research Farm Kalyanpur, Kanpur. The lowest disease intensity amongst the locations surveyed, was recorded at Allahabad University Farm Beli, Allahabad which was more or less similar to Varanasi.

As regards the degree of disease in different types varieties, the maximum disease was observed in bold seeded varieties followed by middle seeded and small seeded varieties.

Seed borne diseases are quite common in Bengal gram crop. The pathogen may be mixed with the seed lot. Since seeds are good substrate for fungi, they build up their inoculum potential on the seeds during germination and even kill the seedlings. Establishment of seed infection is more complicated. Many factors, particularly physiological conditions both of the pathogen and the host in conjunction with weather conditions are responsible for it. A few seed borne pathogens of pulses have been thoroughly investigated with regard to the precise cause of transmission, including establishment of infection and development of the disease in the subsequent crops.

Seeds of Bengal gram often become infected through pods and fruits and injections involve the seed-coat and usually also a considerable part of the cotyledons. The site of infection in the fleshy cotyledons, which form a rich nutritious base for the seedlings as well as far pathogens may essentially play a key role in transmission of these pathogens. According to Neergaard (1977), a disease is borne through the seed, in the sense that potentially it is brought forth or given support by the seed.

The pathogen is an agency which generates suffering in plants and the pathogenesis is the process for the appearance of particular disease and includes the action of pathogen. The susceptibility of the host and the impact of the ancillary factors. That quality or the ability of pathogen to generates disease is the pathogenicity which is an abstract term that carries no implication of how disease is caused. To prove that a certain organism causes a specific disease, it is necessary to establish the Koch's postulates i.e. isolation of suspected pathogen from the infected plant tissues in order to establish the identity in culture. The culture is inoculated on other healthy plants to develop the same type of disease symptoms and re-isolation of organism from the artificially inoculated plants with identical characters.

The infected seeds and infected plants showing the initial and distinct characteristic symptoms, were selected for isolation of the pathogen. The selected leaves were washed with fresh and sterilized water in order to remove the dust particle and surface contaminants. Subsequently, young diseased parts of the leaf were cut into small pieces alongwith some healthy portion with the help of sterilized blade. These pieces were dipped in 0.1% solution of mercuric chloride for 30 seconds

with the help of sterilized water and then washed thoroughly three to four times with sterilized water to remove the traces of mercuric chloride. Excess moisture was removed by putting these pieces pressed in between two folds of sterilized blotting paper under aseptic conditions in the inoculation chamber. These pieces were then transferred with the help of sterilized forceps to petridishes which were already poured with 2% Potato Dextrose Agar medium. As soon as the mycelial growth was visible around these pieces, the hyphae tips from the advancing mycelium were transferred aseptically into the sterilized culture tubes containing 2% Potato Dextrose Agar medium. The culture was purified by single spore technique.

A diluted spore suspension was poured on plain agar in Petri-dishes and spores course allowed to settle down on the agar surface. The amount of suspension was so adjusted as to form a very thin layer over the surface on the agar spored which settled apart from each other were selected under the microscope incurred with the help of dumy cutter in Petri-dish. They were lifted alongwith agar and transferred to Petri-dish already containing sterilized. Potato Dextrose Agar medium. After purification, the cultures were multiplied and maintained at 2% Potato Dextrose Agar slants in the refrigerator at 6-8°C for further studies.

Seeds of Bengal gram was collected from various places and fungi were isolated, purified and maintained on media as mentioned in Chapter II and results are recorded in Table - 2.

A similar study was also carried out with different varieties of Bengal gram crop and the results are tabulated in Table - 3.

(2) Pathogenicity Test

Pathogenicity tests were performed by rolling surface sterilized seeds on sporulating cultures of the isolates and planting them on sterilized moist blatter paper in Petridishes as well as planting them in pots filled with sterilized field soil. Suitable controls were also maintained. Method suggested by Kilpatrick et al., (1954) was used to confirm pathogenicity at seeding stage. Cleaned test tubes with 10 ml. tap water were taken. A sheet of whatman No. 42 filter paper was placed over the end of a wooden plug (which can easily go inside the test tubes). The sides of the filter paper was pressed down and then rolled around the wooden plug. The rolled paper was then pushed down into the test tube, leaving the plat form out of the water. Tubes were plugged with cotton and sterilized. Various isolates were grown on P.D.A. Fungal discs of 5 m.m. were cut, with the help of a cork borer. Such discs were placed at the centre of the filter paper platform. Earlier surface sterilized seeds of desired host were germinated and when their length was about 0.5 cm in length they were asceptically transferred to the filter paper platform. Tubes were labeled and placed on the tube stands for 10 days. Six replicates were taken in each case.

Aqueous mycelial and spore suspensions of the organisms prepared from 7 to 10 days old cultures were sprayed on injured as well as injured flowers and leaves of their respective host plants with the help of hand automizer.

Pods on plants were inoculated by Pin-pricked injury and spraying the spore suspension. Humidity was maintained by covering it with a

polythene bag with some sterilized water at the base. Pluked pods were similarly inoculated and kept under moist glass chambers. Suitable controls were maintained for each treatment. In each case similar conditions such as a mount of inoculum, water and same kind of soil etc. were provided.

Fungi which were found to cause various rots (seed-rot, root-rot, stem-rot. Flower rot and pod-rot) wilt and leaf spots and the symptoms produced by them are recorded in the Table 4.

The same species of *Fusarium* viz., *F. oxysporum* (Ellis and Everh) *F. solani* (Schlecht) which commonly occur in cultivated soil of Allahabad as well as isolated from seeds of Bengal gram crops were taken for pathological studies. These studies were carried out from the same stock cultures and hence the method used for isolating, subculturing etc., were similar to those described earlier. For pathogenicity tests Bengal gram seedlings of same size and age (4 weeks old) were taken pathogenicity tests were carried-out by the following methods.

1. The seedlings root-rot both uninjured and injured (10 injuries by sterilized needle per root) were dipped inspire suspension (about 100 spores per lower field of compound microscope) of *Fusarium* species. The seedlings were then replanted in plastic pots. Controls were simultaneously maintained.

2. The seedlings were kept in culture tubes containing 20 days old culture filtrates of *Fusarium* species. In case of control, seedlings were kept in sterilized distilled water. Daily observations were made.

3. Re-isolations were always made in order to confirm the infection with particular *Fusarium* species. Ten seedlings per treatment were taken in each case. The results of both set of experiments are summarized in the Table 5.

Table : 2

Fungi Isolated from Seeds of Bengal gram (*Cicer arietinum*) Crop.

Crop	Fungi Isolated from untreated Seeds	Fungi isolated from treated seeds
Bengal gram, or Chickpea (*Cicer arietinum*	*Alternaria alternata*	*A. alternaria*
	Alternaria circinans	
	Alternaria tencissima	*A. tencissima*
	Aspergillus Flavus Link	
	Aspergillus flavus	
	A. niger	*Aspergillus flavus*
	Ascochyta - mycosphaerella rabici	
	Botrytis cinerea	*Botrytis cinerea*
	Colletotrichum dematium	*Colletotrichum dematium*
	Curvularia verruculara	
	Curvularia lunta (Wakker) Boedijn	*C. lunata*
	Chaetomium arcuatum	
	Cochliobalus spicifer	
	Fusarium solani (Mart) sacc.	*F. solani*
	Fusarium orthocarpus	*F. orthocarpus*

Fusarium oxysporum	*F. oxysporum*
Fusarium sp.	
Fusarium equiseti	*F. equiseti*
Operculell paduicki	*O. paduicki*
Phyllosticta rabiei	*P. rabiei*
Pythium ultimum	
Penicillium javqnicum Var Beyma	*P. Javanicum*
Penicillium globosum	*P. globosum*
Macrophomina phaseolina	*M. phaseolina*
Rhizoctonia beticola	*R. beticola*
Rhizopus arrhizus	*R. arrhizus*
Sclerotium rosfsii	*Sclerotium rosfsii*
Stimphylium sarciniforme	*S. sarciniforme*
Stachybatrys atra	*S. atra*
Uromyces ciceresarietini	*U. ciceriesarietini*
Xanthomonas cassiae	*X. cassiae*

Table - 3
Fungi Isolated from some varietal samples of Bengal Gram Crop.

Varieties	*Alternaria*	*Ascochyta rabiei* (Pass)	*Botrytis cinerea*	*Colletotrichum denatium*	*Fusarium*
A-Desi Type					
K-468	Al_1+,Al_2+	++	+	++	F_2+++
K-850	Al_1++,Al_3+	+	++	++	F_1+,F_2++
Type-1	Al_1+		+	++	Fu_1++,F_2++
Type-3	Al_2++ Al_3+	+	++	+	Fu_1+F_2++
Pant G 114	A_1+++		+	+	Fu_2+++
Amar (Pusa-203)	Al_1+, Al_2++		+	+	Fu_2+++
Pusa-209	Al_2+	++	+		Fu_1++, F_2+
Pusa-212	Al_1++	+	++	++	Fu_1+Fu_2+Fu_3++
Ajay (Pusa 408)	Al_1++, Al_2++	+	++	+	Fu_1++, Fu_2++
Atul (Pusa 413)	Al_1++, Al_2++	++	+	+	Fu_1++, Fu_2+++
Girnal (Pusa 417)	Al_1+++	++	+	+	Fu_1++
C-214	Al_1++, Al_2+Al_3+	+		++	Fu_1++, Fu_2+
C-235	Al_1+, Al_2+	+	++		Fu_1++, Fu_2++, Fu_3+++
G.C.P. 101	Al_1++, Al_2+	+	++	+	
Gaurav (H-75-35)	Al_1++, Al_2+	++	+	+	Fu_1++, F_3+
B.G.D. 72	Al_1++, Al_2+	+	++		
H-208	Al_1+		+	+	Fu_1++
R.S. 10	Al_1++, Al_2++	++	+	++	Fu_1++, Fu_2+
R.S. 11	Al_1+++	+	++	+	Fu_1++, Fu_2+
B. Kabuli Type					
K-4	Al++, Al_2+	++	+	+	Fu_1++, Fu+++
K-5	Al_2++, Al_3+	+	++	++	F_1++, F_2++

Intensities of Fungal association

+	5-10%	Al_1	*Alternaria alternata*	Fu_1	*Fusarium solani*
++	10-15%	Al_2	*Alternaria circinars*	Fu_2	*Fusarium oxysporum*
+++	15-20%	Al_3	*Alternaria tenuissima*	Fu_3	*Fusarium sp.*

Table : 4

Showing Pathogenicity Results

Crop	Pathogens	Disease
Bengal gram (*Cicer arietinum*)	*Alternaria alternata*	Leaf spot
	Fusarium solani	Seed-rot, seedling rot and wilt
	Fusarium moniliforme	Wilt, seeding rot
	Fusarium oxysporum	Wilt, seed rot, seedling rot
	Fusarium equiseti	Root-rot, wilt
	Fusarium acuminatum	Root-rot, wilt

Table - 5

Pathogenicity test and percentage wilting of Bengal gram (high yielding varieties) at seedling stage

Sl.No	Name of Varieties	Organism	Percentage of Seedling Wilt		
			After 8 days		After 5 days
			Uninjured	Injured	Culture filtrate
1.	K-468	*Fusarium oxysporum*	45	55	65
2.	Amar (Pusa-203)	*Fusarium solani*	40	50	60
3.	Ajay (Pusa-408	*Fusarium oxysporum*	55	60	65
4.	Girnal (Pusa-417)	*Fusarium solani*	50	55	60
5.	Gaurav (H-75-35)	*Fusarium sp.*	46	58	66
6.	H-208	*Fusarium solani*	50	67	74
7.	K-4	*Fusarium solani*	40	50	60
		Fusarium oxysporum	48	59	68
8.	Control		NIL	NIL	NIL

The result from isolation studies showed that in the case of Bengal gram crop, maximum association of *Fusarium* spp. was observed on in the treated as well as untreated seeds. Other fungi isolated from the untreated seeds were species of *Alternaria alternata, Ascochyta rabiei, Botrytis cinerea Colletotrichum dematium* and *Fusarium*

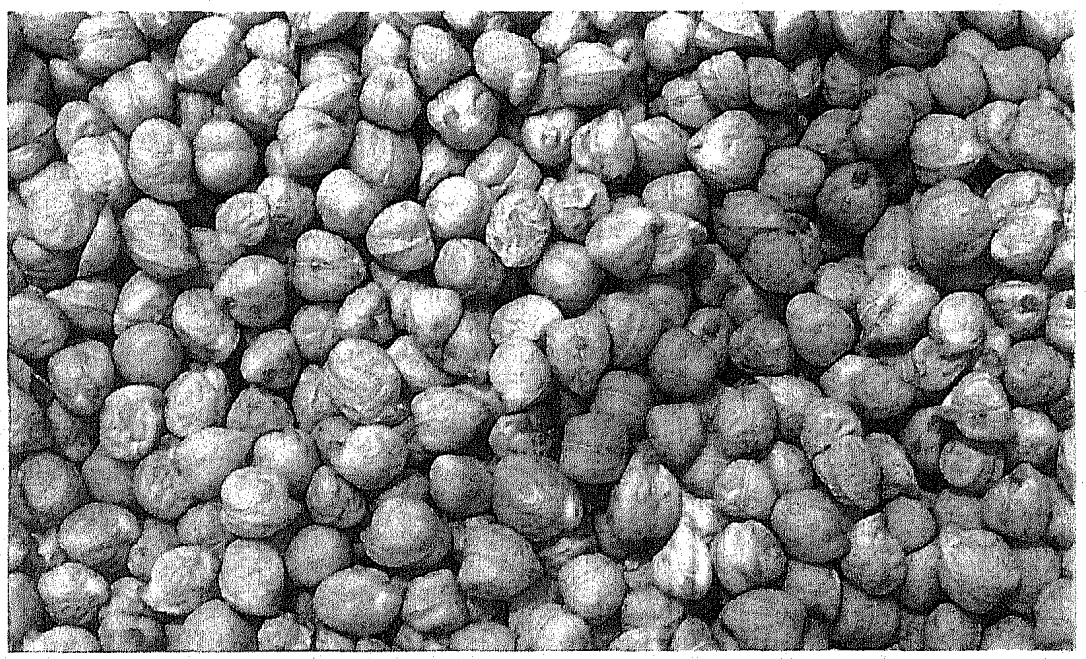
The fungi isolated from known varieties of Bengal gram crop seeds, were similar to those isolated from composite samples of unknown varieties.

Pathological studies revealed that *Fusarium solani* caused seed-rot, pod-rot, seedling rot and wilt of *Cicer arietinum. Fusarium oxysporum* caused wilt, seed rot, and seedling rot of *Cicer arietinum. Fusarium moniliforme* caused wilt and seedling rot of *Cicer arietinum. Fusarium acuminatum* caused Root-rot and wilt of *Cicer arietinum* were also able to cause wilt, rot-rot seed and root rot respectively. In case of root-rot and wilt, infection appears to be seed-borne, as the disease could also occur in infested soil.

Result from the above pathogenicity test clearly show that all the four species of *Fusarium* were capable of causing wilt of Bengal gram and seedling. The main symptom of wilting was as if the seedlings have suffered from water shortage. The wilting was characterized by gradual withering, yellowing and drying of leaves. Later on it was followed by seedlings. It was observed that there was slightly higher percentage of infection increase of injured root seedlings than those uninjured out of *Fusarium oxysporum* was more pathogenic to Bengal gram seedlings than the other two species, as it caused a higher percentage of wilting in the seedling.

CHAPTER - 4

Storage Studies



STORAGE STUDIES

While harvesting of the seeds is seasonal, its consumption in continuous throughout the year, therefore, crop should be stored for few months to at least one year to ensure the continuous supply. It is said that without an ample storage of seeds, there can be no national treasure or no future for the nation.

Protection of seeds is exercised through a complex logistical system that moves seeds from producer to consumer. Quality is of concern throughout the whole system but control is normally practiced where storage facilities are available.

The storability is largely dependent upon the chemical changes which take place in the seeds during storage and is governed by temperature, humidity aeration and microbial activities.

Storage fungi cause great problem during storage. Sick wheat mustiness, heating and caking and binburning, are all due to storage fungi and are products of poor storage conditions. However, to the farmer all this scientific research would be of little value unless he gets seeds which are genetically pure and possess all desired qualities namely, high germination percentage and vigour, high purity, sound health etc. when the farmers do not get seeds possessing these qualities the yields they obtain may not be as expected. In other words the seeds of hope may turn into seeds of frustration.

The scientific method of large scale or bulk storage of seeds is done either in silo elevators which may be made up of concrete or metal bins or in ware-house where the seeds are stored in bags which are kept 6 to 10 inches above the ground on wooden frames. These warehouse may have bolted steel walls with sloping roof or brick-walls with slopping cement asbestos roof and are provided with arrangement for aeration.

Besides the above scientific methods, in India the seeds are also stored at farmer and trader levels in two types of storage structures. The one consists of underground of pits lined from inside with straw ropes and husk and plastered with cow during to prevent seepage of water from surrounding soil. This is commonly known as 'Khattis', 'Matk, 'Kothi', Kuthla', Bukhari', 'Theka' and 'Bharola' with capacity of storing seeds from 2 to 100 quintals each, depending upon the size and shape. There are a considerable variety of structure, depending upon the local conditions and traditions.

Essentially adequate regulation of storage condition is to prevent infestation of insects and keep down humidity on a safe level. In countries with a humid climate such as Indonesia, the traditional system of storage i.e. to put the grain on shelves in the kitchen above the oven is practiced. In South Vietnam grain is stored in cylindrical bamboo frames which are provided with a layer palm leaves around the sides and on the bottom, more over a layer of rice hulls is placed on the bottom and another layer on the top of grain butz. The container is placed in the kitchen. While in Ghana, maize intended for planting is stored in the farm-houses are used for storage. They are placed on a platform made of wood or bamboo. In

India, this practice is also utilized for maize, Jwar, Bajra and Pumpkin seeds. In Upper Volta various types of store huts are used.

Any empty bins store-house, storehut, storepet etc. must be thoroughly cleaned before new grain is stored. Grain debris droppings from birds and mice etc. must be removed and roofs and walls should be inspected, repaired and made waterproof roof.

It is quite possible that the conditions prevailing at the time of harvesting have an influence on the longevity of the seed and seed borne fungi. Therefore close attention should be paid to proper threshing procedures to minimise or eliminate seed injury.

Leguminous seeds are particularly sensitive to thresher injury which in beans may produce upto 30% abnormal seedlings Harter (1930). The epicotyl, being fractured just below the plumule causes 'Balhead' or 'Snakehead' a type of abnormality. Balhead rarely occurs in seed threshed by hand Borthurick, (1932). In cereals damage occurs particularly when the grain is threshed after a dry period of maturation and harvesting Zundel (1921), Wallden (1916) Machak and Grearnedy (1933) and Kocheler, (1957) reported that mechanically injured grains are much more susceptible to molds, saprophytes as well as pathogens, than are uninjured seeds. Alberts (1927), has shown that mechanical damage shorters the storage life of maize seeds.

The air humidity during storage of seed is an essential for keeping the viability of seed. High humidity decreases the germination capacity while the number of abnormal seedlings increases.

Seeds are hygroscopic, and once dried they can later gain moisture from seepage, leakage, and moisture shifts, from production of moisture by insects, mites and fungi and from contact with air of high humidity. Drying of seeds so as to maintain its quality, including high germinability, to avoid breakage in subsequent handling and to achieve a low enough moisture content that will permit storage and shipment without spoilage, is becoming more and more important in national and international trade. In India the drying of grains and seeds, so as to maintain high quality for food and feed is accompanied by so many difficulties. Here farmers dry seeds when it is raining or the weather in hot and humid. The aim in drying grain then is not remove as much water as possible, but to remove as little as possible to meet a given grade, or to make the grain safe for storage for a given length of time, at a given temperature.

According to Papavizas and Christensen (1958), "Wheat with a moisture content upto 16% may be stored without obvious deterioration for a year at a temperature of 10°C or below and wheat with a moisture content upto 18% may be stored safely for as long as 19 months at temperature of 5°C". Qasem and Christensen (1958) working with samples of corn stored in the laboratory at moisture contents of 16% and 18% and temperatures of 5°C, 10°C, 15°C, 20°C and 25°C stated "Low temperature was as effective as low moisture content in preventing damage by the fungi tested".

In the present investigation an attempt has been made to isolate fungi from different seeds stored for different length of time as well as stored in different containers. An attempt has also been made to isolate

fungi from hand, bullock and machine threshed seeds. Effect of seed moisture on seed fungal flora has also been studied.

Different seed samples of Bengal gram (*Cicer arietinum)* stored for different length of time were collected from Agricultural Institute Allahabad, local markets and adjacent villages of Allahabad city. Hand, Bullock and machine threshed seeds were collected from Baberu, Atarra and Village of Banda district.

Fungi isolated from seeds of Bengal gram crops stored at different length of time are summarized in Table - 6.

Effect of threshing and storage conditions on fungal contamination with seeds of Bengal gram (*Cicer arietinum)* is recorded in Table - 7.

Percentage of infested seeds due to various fungi at different range of moisture contents of the Bengal gram crop (*Cicer arietinum)* are recorded in Table-8.

Table : 6

Percentage Infestation of Seeds of Bengal gram (*Cicer arietinum)* for different length of time

Duration	Percentage of infected seeds
Fresh seeds	15.63
Stored for 6 Month	23.04
Stored for 1 Year	19.78
Stored for 2 Year	15.93
Stored for 2.6 Year	21.09
Stored for 3 Year	12.02

Table - 7

Showing Effect of Threshing and Storage Condition on Seed Infestation of Bengal gram (*Cicer arietinum)*

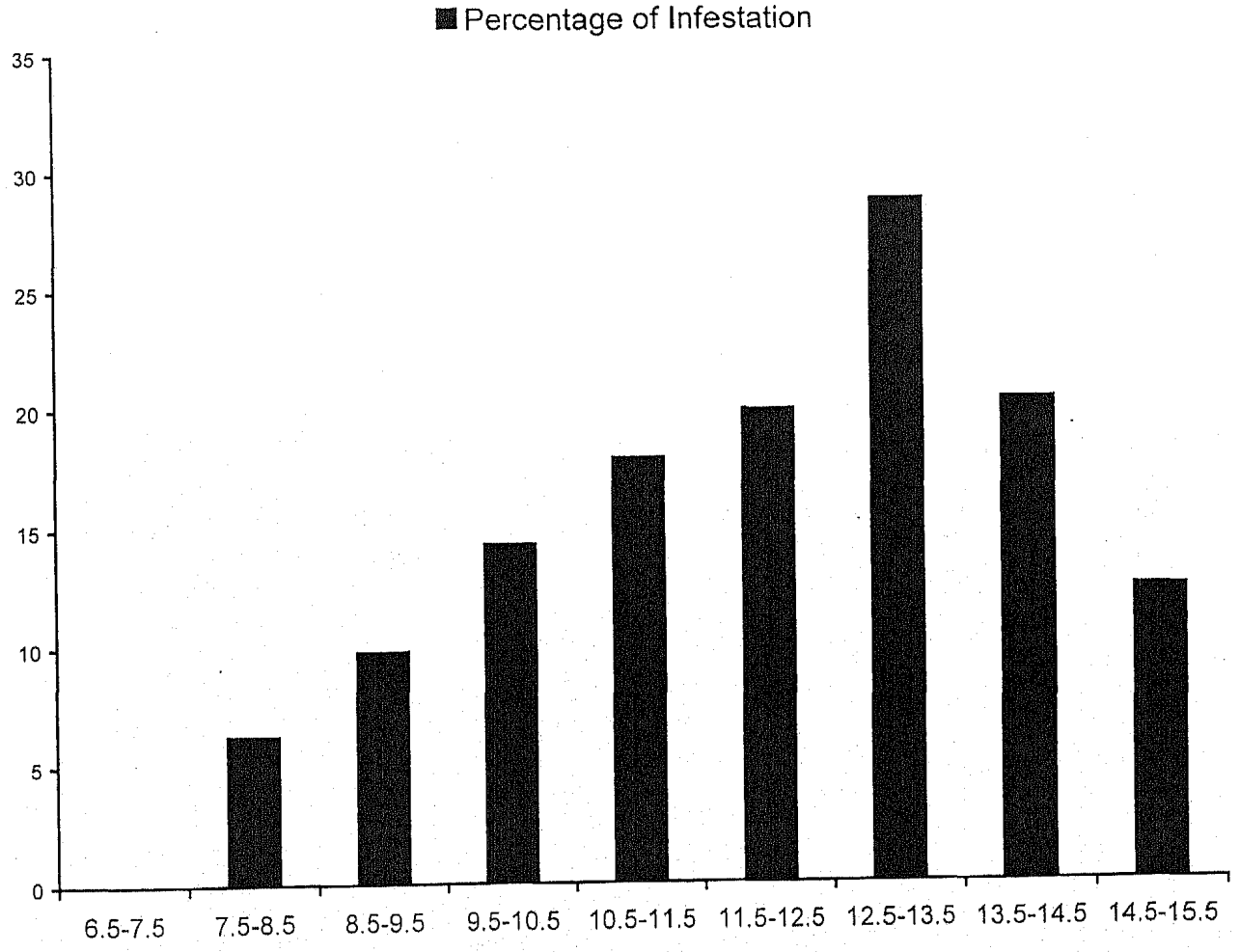
	Percentage of Seeds Infestation
Threshing Methods	
1. Machine	23.00
2. Bullock	14.00
3. Hand Threshed	10.00
Collection Sources	
1. Tin Container	11.00
2. Kathila	13.00
3. Under Ground Pits	16.00
4. In Bags Covered with Wheat Straw	11.00
5. On Floor	16.00
6. Earthen Pots	16.00

Table - 8

Showing Percentage Infestation of Seeds of Bengal Gram (*Cicer arietinum)* as Different Range of Moisture

Percentage of Seed Moisture Range	Percentage of Infestation
6.5-7.5	0.0
7.5-8.5	6.25
8.5-9.5	9.82
9.5-10.5	14.34
10.5-11.5	17.89
11.5-12.5	19.92
12.5-13.5	28.78
13.5-14.5	20.35
14.5-15.5	12.45

Showing Percentage Infestation of Seeds of Bengal Gram *(Cicer arietinum)* as Different Range of Moisture



CHAPTER - 5

Biochemical Studies

BIO-CHEMICAL STUDIES
(LOSS IN PROTEIN CONTENT)

Nitrogen is an essential element for fungi. It has structural as well as functional importance and occurs in fungi in the form of complex organic nitrogenous compound. According to Foster (1949) the nitrogen content of mycelium lies in between 3% and 6% which varies with the species, age, nutrition and other factors.

Among the organic sources of nitrogen, amino acids influence the growth of various fungi. Amino acids are assimilated directly by most fungi and are incorporated in to the protein. A number of fungi are thus found to be associated with seeds of Bengal gram which are rich source of protein. They in storage may cause the loss or degradation of these protein.

Spore suspension of four seed borne fungi viz. *Fusarium oxysporum, Fusarium solani, Fusarium moniliforme and Fusarium semitectum* were sprayed on the surface sterilized seeds of Bengal gram and incubated at room temperature (22 ± 1°C). After 15:30 and 45 days of incubation, protein content of seed was determined. The total nitrogen was determined by digesting 100 mg of oven dried and finally powdered seed materials of Bengal gram, in 50 ml micro-kjeldahe flask. The reagents used were similar to as suggested by Peach and Tracey (1955)

Reagents : Catalyst : 1 gm copper sulphate 8 gm potassium sulphate, and 1 gm selenium dioxide

All of these were weighed separately and mixed together with mortar to form the catalyst powder.

Indicator

It consisted of 6 ml of methyl red (0.16% in 95% alcohol) 2 ml of Bromo-cresol green (0.04% in water) and 6 ml 95% alcohol, when it was added to distillate it turned green but at the end point (pH 4.9) it gives faint pink colour.

N/28 Hydrochloric Acid

This was prepared by diluting 31.8 ml of reagent grade HCL to 10 litres with glass distilled water. Since there may be differences in the quality of HCL used if they are prepared from different sources, it was considered necessary to prepare sufficient amount of HCL at one time which keeps indefinitely and this was carefully standardized against freshly prepared N/28 NaOH (Standardized against N/28 Oxalicacid).

PROCEDURE

Digestion

100 mg of dry powdered seed material was weighed on a small piece of fine-quality tissue paper and carefully transferred to a micro-kjeldahl flask. Small amount (about 0.06 gm) of catalyst was added by means of paper which served as spatula; this avoided any particle adhering to the sides of neck of the kjeldahl flask. The kjeldahl flasks were thoroughly dried before use. About 1 ml of nitrogen free conc. H_2SO_4 was also added. In practice the catalyst and H_2SO_4 were added before the plant material was introduced into the flask. The flask were heated gently on a digestion stand in a fume cupboard, until fumes of sulphuric acid are freely evolved and then heated more vigoursely until digest was apple-green in colour. Digestion was continued for at least half an hour more. Blanks on the reagents alone were run at the same time. During digestion great care was taken to avoid particles of undigested material sticking to the sides of the flasks.

Distillation

The apparatus was thoroughly steamed out before use with water boiling vigorously in the flask, the clip above the flask was closed so that the steam passed into the steam jacket. The digest was introduced into the apparatus through the funnel from the side and digestion flask was washed twice with distilled water (1 ml each time). The funnel was washed with 3 ml of distilled water before NaOH was added. About 10 ml of 40% NaOH was added through the funnel. The clip before the funnel was then closed

and steam was allowed to pass freely into the distillation flask. The ammonia evolved passed through a condenser whose lower part was dipped in a 50 ml conical flask containing 50 ml of 2% boric acid. At the commencement of the distillation, the top of the condenser was raised above the bottom of the flask. Distillation was continued until about 20 ml of distillate had collected. When distillation was completed the conical flask was removed and the lower lip of the condenser washed into this flask with distilled water. The outlet of the flask in which water boils was then opened and the clip which allowed the steam to pass into the distillation flask was closed. This resulted in lowering of the pressure in the distillation flask and the liquid in flasks automatically into other flask placed in between the distillation flask and the steam generating flask, water was poured through the funnel and this washed through into the steam jacket. The clip of this flask was opened and the waste allowed to run through the bottom hole. The apparatus is then ready for another distillation.

Two drops of indicator were added to the distillate and then titrated with N/28 HCl to a faint pink colour. The amount of HCl required was multiplied by 0.5 to get mg of total nitrogen per 100 mg of dry seed material since 1 ml of N/28 HCl was equivalent to 0.5 mg of N. The total protein was calculated by multiplying the total nitrogen by 6.25. The loss in protein content of seeds infested Bengal gram are recorded in Table - 9.

Table : 9

Showing the effect of *Fusarium oxysporum, Fusarium solani, Fusarium moniliforme* and *Fusarium semitectum* on the protein contents of seeds of *Cicer arietinum*

Fungi	Control	% Protein content of Bengal gram seeds		
		15th day	30th day	45 day
Fusarium oxysporum	21.1	20.56	19.78	18.92
Fusarium solani	21.1	20.53	19.21	17.89
Fusarium moniliforme	21.1	20.12	18.43	16.35
Fusarium semitectum	21.1	20.04	18.04	16.12

Result from Table-9 show that the protein contents of inoculated seeds of Bengal gram *Cicer arietinum* with *Fusarium oxysporum* and *Fusarium solani* either remained unchanged or were a little altered during first 15 day which obviously reflects the slow fungal activity in the beginning. There was a gradual depletation of protein in the seeds from the second phase which continued upto the end of the incubation period. Seeds incubated with *Fusarium moniliforme and Fusarium semitectum* showed remarkable reduction in protein contents, after 15 days of incubation. Seeds of control sets from initial period upto the end of 45 days of incubation did not show any reduction in the protein content.

CHAPTER - 6

Survival Studies

SURVIVAL STUDIES

Seed and soil are resting places for a wilt pathogens and it is a primary source of inoculum for onset of plant disease. Our knowledge regarding factors affecting survival of various wilt and root-rot causing organisms is, however, very meager. A number of seed factors like temperature, moisture or amendment affect the population of pathogen and thus regulate the severity of infection. Creating unfavourable conditions for the pathogens either by regulating the temperature seed moisture or by amending the seed soil with chemicals will help in controlling many diseases.

The present study deals with some factors affective survival of three species of *Fusarium* viz. *Fusarium solani, F. oxysporum* and *F. equiseti*, isolated from seeds of different places. Though the effect of various microbiological factors on different seed fungi including ecology and pathogenicity have been studied by many investigators yet a little attention have been paid towards the above aspect. However, many workers including Garrett (1944, 1956, 1963, 1970) Butler (1953), Stover (1953 ab) Newcombe (1980) Papavizas and Davey (1965), Sen Gupta and Ray (1971) as well as Dhingra and Sinclair (1976) have extensively reported the saprophytic behaviour of fungi in soil.

Temperature, humidity and other aspects of the physio-chemical conditions of the soil, the edaphic factors. Particularly soil reaction, soil type and soil fertility are important environmental factors. In the period prior to infection these factors. Exercise influence on the predisposition of the

host and the preparedness of the pathogen. If the environment favourable for the pathogen is the same, as far the host, disease incidence may be very high. But it is also possible that the host thrives as such a temperature and humidity which is not favourable for the pathogen. This dissimilarity in suitabilities of environments for host and pathogen can be exploited for disease control. One of the methods to achieve this is to alter the date of sowing so that the susceptible stage of plant growth does not go inside with the environment that favours the pathogens.

According to Manzies, 1963, By far the most common non host environment for organisms in the soil, which, indeed, is the final resting place for all terrestrial life. There fore, the capabilities possessed by crop disease organisms for surviving in a soil environment are of great importance to plant pathology. He wrote further that the plant pathogens, after parasitizing the host plant, may enter the soil in or on host tissue or free in the form of propagative or resting structures.

The present study deals with some factors affective survival of three species of *Fusarium* viz., *Fusarium solani, F. oxysporum* and *F. equiseti*, isolated from soils of different field. Though the effect of various microbiological factors on different soil fungi and seed fungi including ecology and pathogenicity have been studied by many investigators yet a little attention have been paid towards the above aspect. However, many workers including Garrett (1944, 1956, 1963, 1970) Butler (1953), Stover (1953 a,b), Newcombe (1980), Papavizas and Davey (1961), Sen Gupta and Roy (1971) as well as Dhingra and Sinclair (1976) have extensively reported the saprophytic behaviour of fungi in soil.

In the present study Agricultural farm Allahabad University, Allahabad was selected for survival studies. The soil was dried, and sieved and was infested with 3% maize meal sand culture. This was prepared in 250 ml conical flasks. Roch flask was first filled up by 150 gm of sand and maize meal mixture (150 gm of dry clean sand + 4.5 gm of maize meal) and 20 ml of distilled water was carefully added (100 gm dry sand holds 20 ml water at saturation so 20 ml for 150 gm sand, maize meal mixture gives about 65% saturation). Such flasks were then autoclaved for 30 min at 20 lbs pressure and were inoculated with agar inoculum discs from a colony margin of a 7-10 days old culture of the fungus on Potato Dextrose Agar. Flasks were incubated for about 4 weeks at 25° ± 1°C and were shaken after 2 weeks to distribute the fungus.

When the above flasks containing 3% maize meal cultures of *Fusarium* species were well grown they were then ready as inoculum for infesting the soil of glass jars. 5 gm of the inoculum (maize meal sand culture) was mixed with 100 gm of unsterilized air dried soil. To assist in the distribution of inoculum the jars were thoroughly shaken and 20 ml of water was added to give 50% saturation of soil. Such jars were weighed and weights were recorded on the jars. To maintain moisture content of 50% saturation all the jars were weighed on the pan-balance about twice a week and distilled water was added carefully until the original weight was restored. The inoculated glass jars were kept in the laboratory and covered by the petridishes halves to reduce moisture loss. The small gaps served the purpose for air exchange. Five jars per treatment were taken. For the survival studies jars were shaken well before taking out the soil samples.

The method of isolation of *Fusarium* colonies was similar to those described earlier.

For the study of effect of temperature on the survival of the present three species of *Fusarium* the soil was infested with respective *Fusarium* species in the glass jars. They were incubated at a temperature ranging from 5° to 45°C. After 7 days of incubation, isolations were made on usual. The results are recorded in the Table - 10.

The maximum survival of all the *Fusarium* species was observed between 20° and 25°C. Their numbers (colonies) decreased as the temperature was either increased or decreased. Maximum number of colonies of *F. equiseti* and *F. oxysporum* were observed at 25°C while in case of *F. solani* maximum number of colonies retrieved was at 20°C. At 5°C, and 40°C the survival of the present fungi was minimum. No colonies, however, could be recovered at 45°C.

Sen Gupta and Roy (1971) found the optimum maximum soil temperatures for competitive saprophytic survival of *Sclerotium rolfsii* was between 20°C and 30°C. The similar results were also obtained by Gondo (1969) for the growth of *Sclerotium rolfsii*.

Various investigators including Blair (1943), Staver (1953 a,b) Newcombe (1960), Sen Gupta and Roy (1971) and Sinclair (1975) have reported the effect of soil moisture on the saprophytic activities of a number of fungi studies by them. In the present investigation the effect of soil moisture on the survival of three species of *Fusarium* was assayed in soil

in which the water holding capacity was adjusted at different percentage ranging from 10 to 100 percent. The results are recorded in Table - 11.

The survival of the present *Fusarium* species was maximum when soil moisture was maintained at 30% water holding capacity. However, above and below 30% water holding capacity of the soil, the survival of *Fusarium* species declined and at 90 to 100% water holding capacity it was almost eliminated. Stover (1963 a) reported earlier, the optimum growth and survival of six species and forms of the genus *Fusarium* in soil at 15% saturation.

According to Sen Gupta and Roy (1971) in unsterilized soil, maximum saprophytic activity of *Sclerotium rolfsii* occurred at a wide range of relative low moisture (20-50% moisture holding capacity). Similarly Blair (1943) and Papavizas and Davey (1961) reported the effect of soil moisture on the saprophytic survival of *Rhizoctonia solani.* The reduction in population at high soil moisture was attributed by Blair (1943) to a decline in soil creation with an increase in mixture content.

The present study indicated that the three species of *Fusarium* were strongly aerobic and their population can be reduced by maintaining the soil in a saturated condition. This view is also supported by Stover (1953a).

Garrett (1938 b) reported a decline in the viability of the mycelium of *Ophiobalous graminis* in soils amended with glucose or with other materials having a little or no nitrogen. According to Wrest and Hildebrand (1941), the incorporation of glucose in soil eliminated the fungi involved in straw berry root rot.

Soil samples were amended with glucose, sucrose and starch. The different carbon sources were added at a rate equivalent in carbon to that provided by 1% glucose in air dried soil (W/W). Non-amended soils served as control. All soil samples were then adjusted to 30% water holding capacity of the soil on an oven-dry weight basis. Samples were incubated at 22°C ± 1°C for 4 weeks. The results are recorded in the Table - 12.

Results from above table show that in general. There was marked decline in population *Fusarium* species in soil amended with glucose, sucrose and starch as observed upto 4 weeks.

In soil samples amended with glucose the population of *F. solani* and *F. equiseti* increased in first week and then declined by the end of 4th week, as compared to control, while there was a continuous decrease in case of *F. oxysporum*.

Among soils amended with different sources the maximum reduction was recorded with sucrose. The population of all the three species of *Fusarium* declined continuously upto 4 weeks starch amended soils had the similar inhibitory effect on the *Fusarium* species population but in case of *F. oxysporum* the inhibition was a little less as compared to sucrose.

The nitrogen sources viz., ammonium sulphate, sodium nitrate and urea were added to give 0.92 N/g (w/w) of air dried soil. The fungal inoculum was incorporated in the soil just after the addition of nitrogen sources. Non-amended soil serves as controls. All soil samples were then adjusted to 30% water holding capacity of the soil and the glass jars were

incubated at 22° ± 1°C for 12 weeks. The results are summarized in the Table - 13.

The population of *F. equiseti* decreased rapidly in soils amended with ammonium sulphate, while in case of *F. oxysporum and F. solani*, it increased upto 2 weeks and then continued to decline till 12 weeks. The former resembled with *Rhizoctonia* studied by Davey and Papavizas (1960).

When the soil was amended with sodium nitrate, the population of *F. equiseti* first increased upto 2 weeks then declined, whereas, in case of *F. solani* the decrease of population was continuous upto 12th week. Similar results were also obtained by Garrett (1971) and them. The population of *F. oxysporum*, however, continued to increase upto 12 weeks. Garrett (1974) also reported similar results for *F. culmorum* and *Casumann omyces graminis.*

The maximum decline of *Fusarium* species population was observed in soil samples treated with urea. In case of *F. oxysporum* population increased upto 2 weeks otherwise, there was a gradual down fall in fungal population upto the end of incubation period. The present results resembled with *Fusarium oxysporum, F. cubenae* studied by Sequeired (1962, 1963).

In control, *Fusarium* species continued to increase upto the end of incubation period (12 weeks) this increase was more rapid in case of *F. oxysporum* and *F. solani.*

According to Cochrane (1958) the possible interaction between carbon and nitrogen sources added be considered first because in some cases it may be necessary to study all putative carbon sources in media with different nitrogen sources. Asthana and Hawker (1936) reported that dilution of the entire medium or of glucose only, accelerated the development of perithecia in Melampsorerdestruens. Reduction of nitrogen level did not influence perithecial development, Baker (1931), however, found that low concentration of carbon favoured the zygospore production in Sporodinia grandis when nitrogen content was increased. Talley and Blank (1941) observed that if carbon source was not limiting factor. The rate of growth was controlled by the concentration of nitrogen but if carbon was a limiting factor, there was no significant increase in the growth of *Rhymatatrichum omnivorum* by increasing the amount of nitrogen. Buston and Basu (1948) and Mathur et al., (1950) found that the growth and sporulation of *Chaetomium glabosum* and *Colletotrichum, lindemuthianum* respectively increased with increasing amount of carbon upto a limit after which the growth increased while sporulation decreased.

The carbon and nitrogen ratio may also influence the type and form of spores produced. Nitimargi (1953) observed that the spores of *Phomopsis* and *Diaporthe* vary from small and oval to large and curved or elongated and sickle shaped, as the ratio of glucose to asparagine is increased. Cultural characters are also reported to be influenced by increasing supply of carbon. Pine (1958) also observed some changes in characters of four isolates of *Phomopsis veticola* when subjected to increasing carbon supply.

Sometimes a low C/N ratio may be harmful because it has been observed that in the presence of low carbon supply, a decrease in the population or root pathogens under a high C:N ratio in the soil has been demonstrated in several instances. Snyder et al. (1959) controlled the bean root rot caused by *F. solani, F. phaseoli* with soil amendments having high C:N ratio. Similarly, Dhingra and Sinclair (1976) also deserved a decline in the sclerotium population of *Macrophomina phaseolina* at high C:N ratio soils were amended with glucose and sodium nitrate in different C:N ratios. Amendments to provide C:N ratio of 10,20,40 and 80 were based on 1% glucose in air dried soil (W/W) and sodium nitrate. The soil samples were incubated for 4 weeks. Results are summarized in the Table - 14.

The population of all the three species of *Fusarium* declined in the soil samples amended with glucose and sodium nitrate mixture. It was also observed that as the C:N ratio was increased, the number of colonies declined more rapidly in the soils. The population of *F. equiseti* and *F. oxysporum* for all C:N ratios decreased in comparison with control upto the end of 4 weeks. The population of *F. solani* increased during the first week then declined upto the end of incubation period.

Table : 10

Effect of Temperature on the Survival of *Fusarium* species

Temperature (°C)	Number of Colonies per 50 mg of Soil		
	F. solani (I)	*F. oxysporum (II)*	*F. equiseti (III)*
5	04	02	06
10	32	24	28
15	43	47	55
20	59	71	66
25	50	67	72
30	45	52	65
35	21	18	33
40	04	02	06
45	00	00	00

Effect of Temperature on the Survival of *Fusarium* species

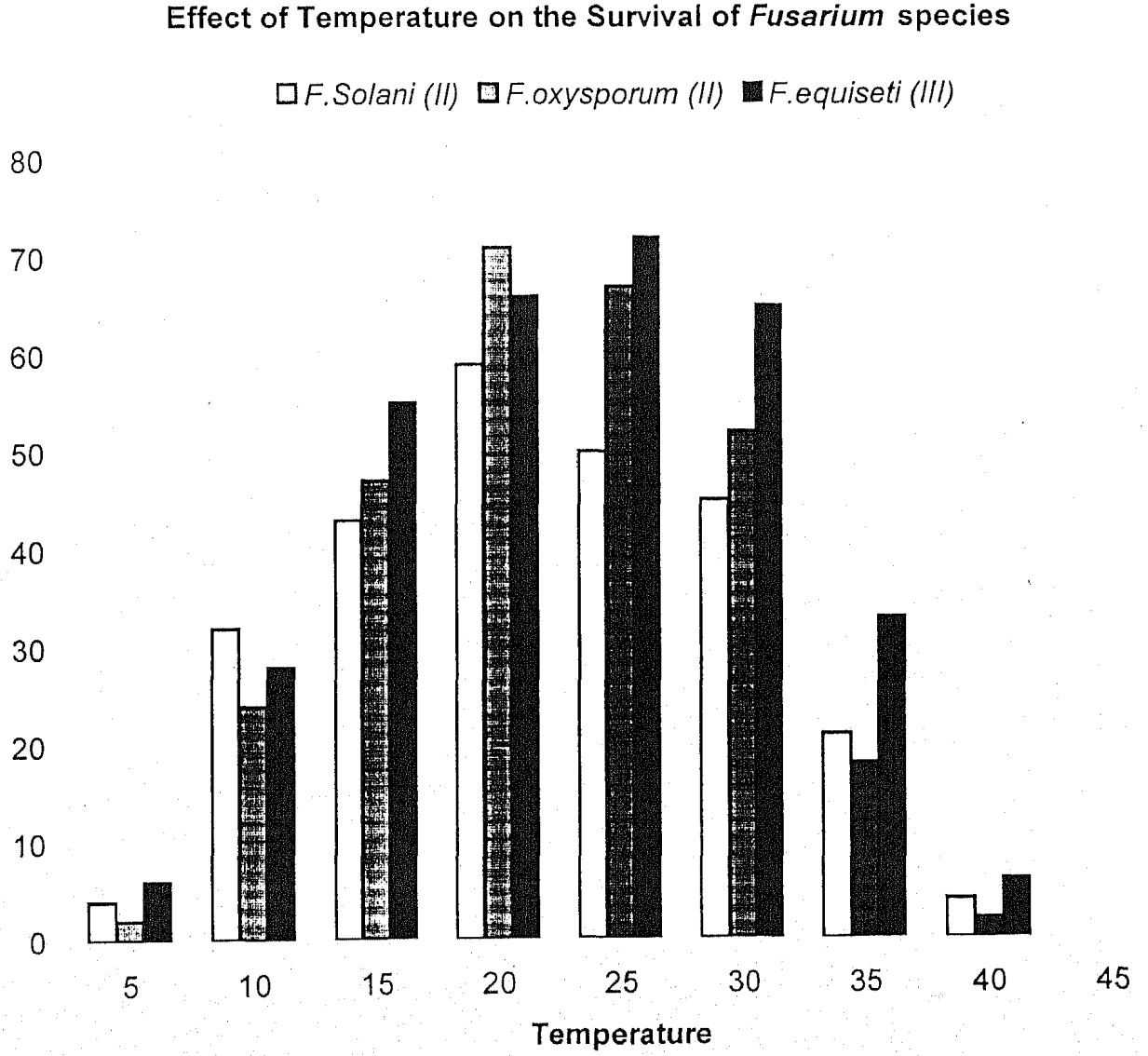


Table : 11

Effect of Soil Moisture on the Survival of *Fusarium* species

% of water holding capacity of (soil).	Number of Colonies per 50 mg of Soil		
	F. solani (I)	*F. oxysporum (II)*	*F. equiseti (III)*
10	35	27	31
20	50	47	48
30	72	73	75
40	43	47	41
50	27	36	31
60	21	26	25
70	15	17	13
80	11	13	15
90	07	09	08
100	05	04	03

Effect of Soil Moisture on the Survival of *Fusarium* species

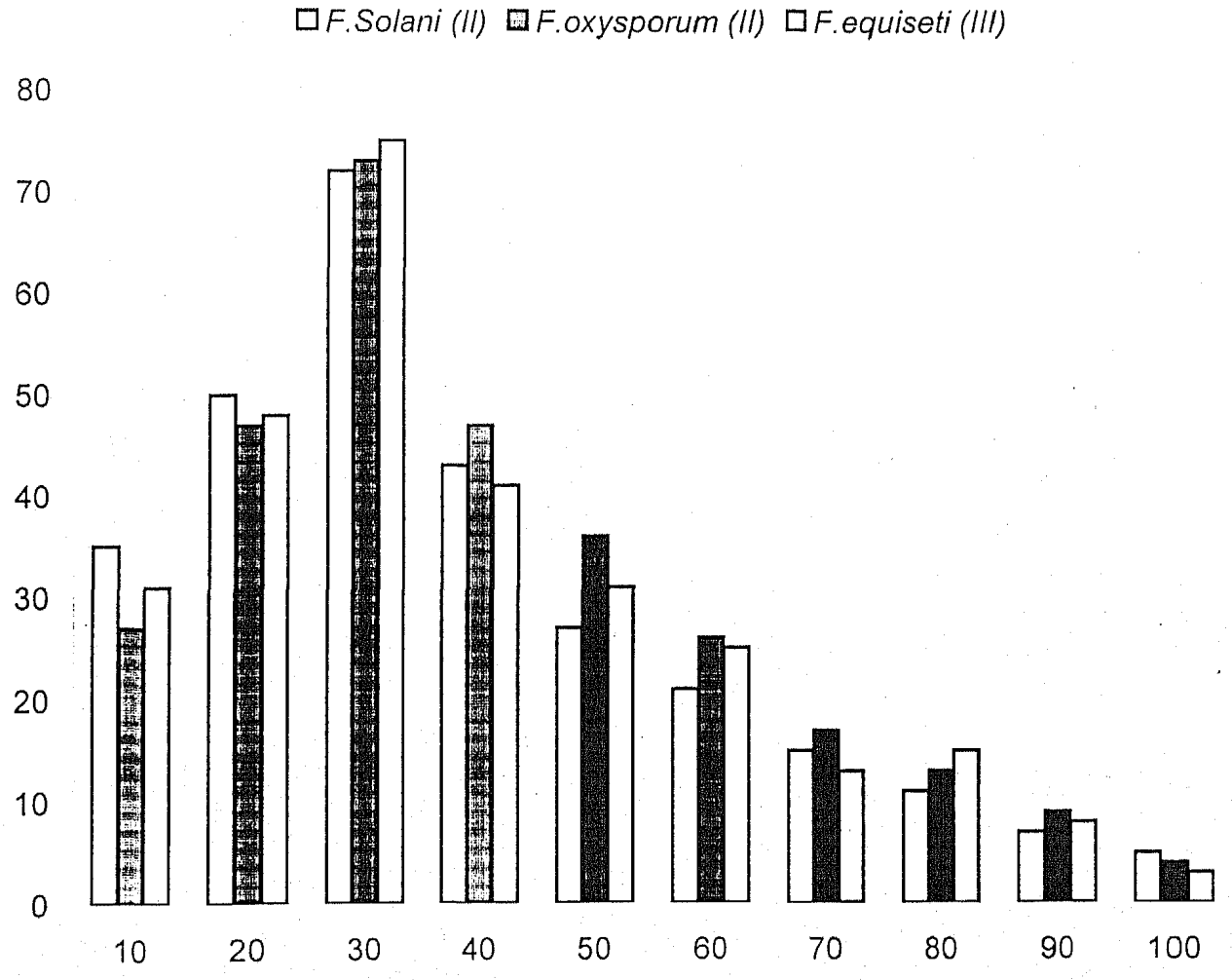


Table : 12

Effect of Carbon Sources on the Survival of *Fusarium* species in Soil

Fusarium species	Treatments	Number of *Fusarium* Colonies after		
		1st Week	2nd Week	4th Week
F. solani	Glucose	96	36	03
	Sucrose	60	38	16
	Starch	30	19	15
	Control	62	63	67
F. oxysporum	Glucose	25	12	04
	Sucrose	07	02	00
	Starch	28	16	08
	Control	72	74	86
F. equiseti	Glucose	78	46	08
	Sucrose	72	13	06
	Starch	27	12	02
	Control	77	53	77

Table : 13

Effect of Nitrogen Sources on the Survival of *Fusarium* species in Soil

Fusarium sp.	Amendment	Number of *Fusarium* colonies after				
		1st Week	2nd Week	4th Week	8th Week	12th Week
F. solani	Ammonium sulphate	58	52	24	28	27
	Sodium nitrate	81	79	67	26	22
	Urea	42	35	31	22	09
	Control	70	49	72	71	87
F. oxysporum	Ammonium sulphate	106	125	41	22	12
	Sodium nitrate	122	131	152	161	185
	Urea	132	118	84	41	31
	Control	72	74	94	122	142
F. equiseti	Ammonium sulphate	62	55	52	29	28
	Sodium nitrate	52	49	47	42	39
	Urea	27	26	22	21	15
	Control	58	56	67	66	132

Table : 14

Effect of C:N Ratios on *Fusarium* species population in Soil

Fusarium species	Treatments C:N Ratio *	Number of *Fusarium* Colonies after		
		1st Week	2nd Week	4th Week
F. solani	10	82	41	16
	20	55	34	18
	40	38	22	16
	80	17	17	01
	Control	59	58	62
F. oxysporum	10	12	07	02
	20	08	06	06
	40	03	01	00
	80	01	00	00
	Control	80	80	95
F. oxysporum	10	30	15	04
	20	23	07	07
	40	07	14	09
	80	01	00	00
	Control	70	50	73

* Based on 1% Glucose in air dried soil (w/w) and sodium nitrate.

CHAPTER - 7

Control Studies

CONTROL STUDIES

"When we know how the parasite breathes, feeds, excretes and uses within its host and at its host expense, whatever biochemical process link it to that host, we may learn it to control it."

— Anonymous (1951)

The nature of Bengal gram wilt disease is seed and soil borne. Therefore, major emphasis for the control of this disease remained through resistant varieties. As a result, very little information is available on the chemical control of this disease.

Pedwick (1941), found that application of farmyard manure hastened the disappearance of the fungus in the soil. Ibragimov, et al. (1966), observed that dipropyl fungicide and fungifas effectively controlled the wilt disease in Bengal gram. Kuzmina (1966), reported that seed treatment of Bengal gram with 10.2 and 0.5 phyto-bacterionycin at 0.001, 0.01 and 0.02 percent and disting with 1 and 26 tricho thecin increased seed germination by 5 to 10 percent, Improved yield and reduced the incidence of *Fusarium oxysporum.*

Chandra and Tomar (1973) suggested that fungicide application in the soil might be helpful in controlling the disease. Khare et al. (1973), suggested that the best method to combat the wilt disease is the use of resistant varieties. These workers observed that late sowings resulted in

reduced in incidence of wilt in Bengal gram. Soaking of seeds for 5 minutes in 0.01% Ceresan wheat followed by seed treatment with 0.25 Thiram and 0.2% PCBB by weight Bengal gram the incidence of wilt to a considerable extent.

(i) Sinha (1975) observed that amendments of soil with oil-cakes of groundnut sesame and mustard reduced the percent mortality of Bengal gram due to wilt, from 63% in control to 21, 18 and 8 percent respectively. Suhag (1973) obtained the best control of the fungi causing pre and post emergence coat of Bengal gram seeds through treatment with Agrosan G.N., Agallal, Septon and Thiram. Singh (1976), that the growth of the pathogen. *F. oxysporum, F. sp. ciceri* was significantly inhibited due to immature and mature wheat, oats, Bengal gram, pea and Lentil crop resides. However, decrease in population and reduction in infection were observed to be non-significant.

(ii) To prevent Bengal gram crop diseases effectively, a knowledge of their nature and cause is fundamental to successful control. Once the causal agent has been correctly diagnosed then it is possible to prescribe effective methods for its control. However they may be prevented by a combination of plant protection principles that are necessary for effective protection against the several disease causing agencies. The following methods are effective to control the crop diseases caused by fungi, bacteria, nematodes, viruses and nutritional deficiencies.

(i) Adopting appropriate cultural practices which would either enable a plant to escape from disease or reduce the inoculum potential of pathogen.

(ii) Restricting the movement of diseased crop material from one region to another through legislation.

(iii) Growing disease resistant varieties.

(iv) Using chemicals to save the plant from disease.

A seed-borne pathogen has greater capacity for spreading in to growing crops than pathogens other than seed-borne. As the primary infection comes from the infected seeds, experiments were carried out to control seed-borne fungi by various fungicides.

Seed borne infection is observed in seeds from plants which wilt after pod formation. Rouging wilted plants at harvest may help reduce chances of seed transmission. Since the pathogen is a soil inhabitant and can survive in the soil for a long time. It is important to eliminate the chances of introducing it through seeds. Howare at al. (1978) demonstrated a mixture of 30% benomyl and 30% Thiram) can completely eradicate seed-borne *F. oxysporum F. sp. ciceri.* It is very important to treat the seeds to stop spread of the pathogen through seed and to facilitate the international movement of the germplasm and breeding material needed for Bengal gram improvement work.

Many workers including Valaska (1962), Sen Gupta and Ray (1971) and Ilyas et al. (1975) have tested a number of Fungicides for soil

disinfection. In view of this fact, a number of fungicides were tried to check the population of *Fusarium* species in soil and seeds.

In a preliminary test, the efficiency of a number of fungicides viz. Aureofungin (a heptathe antibiotic), Benlate (Methyl - I - Butylearbamoy 1) 2-Benzimidazole carbonate), Brassicol (Pentachloronitrobenzene), Cercobin (Benzene thiophanate), Difolatan (cis-N0C1,1,2,2 - tetrachloroethyl), Thio-4-cyclo-hexene-4, 2-dicarboximidie), Ferbam (Ferric dimethyldithio carbamate), Plantvax (DCMOD, 2,3 dihydro-5 carboxa-nilido-6 - methyl-1, Oxathin-4, 4 dioxide), Thiram (tetramethyl thiruram disulphide or bis (dimethyl-thio - carbomoyl) disulphide) and Vitavax (DMOC, 5, 6-dihydro-2-methyl-1, 4 - oxathin-3-carboxanilide against three species of *Fusarium* was tried in vitro. The fungicides were added to the basal medium at different concentrations (50-1000 ppm) and flasks were steam sterilized for 3 successive days for 30 min. Results are recorded in Table - 15. The results show that Benlate at 50 ppm, Cercobin at 500 ppm, Difolatan 100 ppm. Plantvax at 500 ppm and Vitavax at 1000 ppm were found to be inhibitory to the growth of present organisms whereas, rest of the fungicides including the antibiotic, Aureofungin failed to inhibit the growth of present *Fusarium* species even at the concentration of 1000 ppm. (Maximum concentration taken). The concentrations of fungicides which inhibited the growth of the organisms were then employed to check the population of *Fusarium* species in soil. In addition to this, Tecto '40' (Thiabendazole - 42.8%), 2 - (4-Thiazolyl - benzimidazole) was also used.

All the fungicides were amended in the soil at a concentration which was found to be inhibitory to the growth of the organisms in culture media. Tecto '40' was amended to the soil at a concentration of 500 ppm. The

fungal inoculum was incorporated in the soil after 24 hours of addition of the fungicide.

The results are summarised in the Table - 16. Difolatan was found to have a marked retarding effect on the survival of all the three species of *Fusarium*. At a concentration of 100 ppm. The fungal survival was almost eliminated. The incidence of survival of *F. equiseti* and *F. solani* was slightly increased in case of Vitavax and Cercobin respectively as compared to other fungicides Tecto '40' was qualitatively less effective than Benlate in reducing the population of present species of *Fusarium*. Similar results were also obtained by Ilyas et al. (1976) for *Macrophomina phaseolina*.

Table : 15

Effect of Fungicide amendment in soil on *Fusarium* species population

S.No.	Fungicide	Concentration (ppm)	Number of colonies per 50 mg of soil		
			F. solani	*F. oxysporum*	*F. equiseti*
1.	Benlate	50	18	04	07
2.	Cercobin	500	44	07	21
3.	Difolatan	100	00	00	03
4.	Plantvax	500	19	11	15
5.	Tecto '40' (T.B.Z. 42.25%)	500	21	06	11
6.	Vitavax	1000	17	03	42
7.	Control	--	51	73	69

Effect of Fungicide amendment in soil on *Fusarium* species population

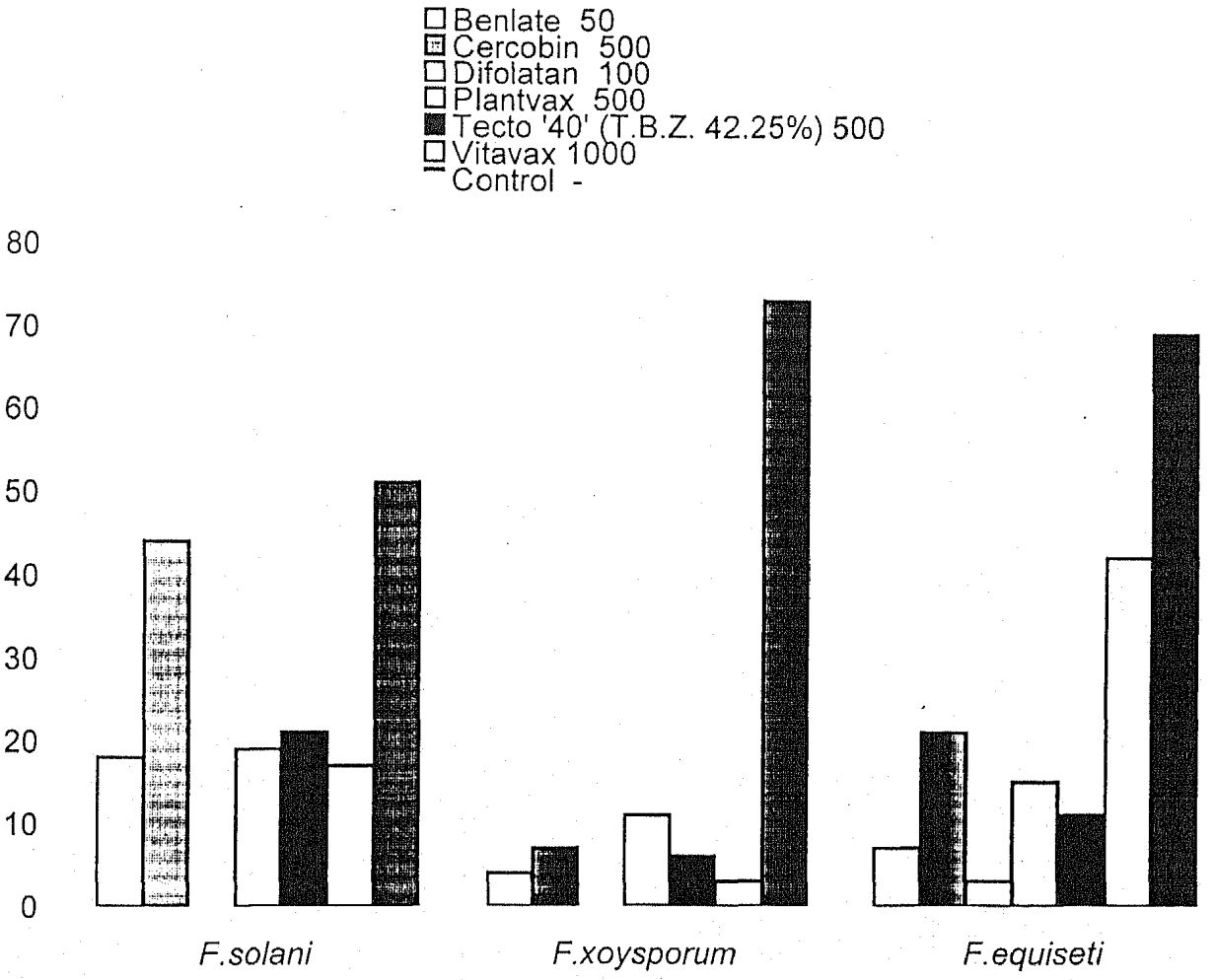


Table : 16

Effect of Some Fungicides on Growth (Dry Weight) and Sporulation of *Fusarium* species

Fungicide	Concentration (ppm)	*F. solani*		*F. oxysporum*		*F. equiseti*	
		dry wet. in mg	sporulation	dry wet. in mg	sporulation	dry wet. in mg	sporulation
Aureofungin	50	64.2	Good	56.2	Poor	55.8	Poor
	100	44.3	Poor	35.8	--	47.0	Poor
	500	27.2	Poor	16.4	--	27.3	--
	1000	10.5	--	7.9	--	9.8	--
Benlate Brassical	50	--	--	--	--	--	--
	50	66.0	Excellent	64.6	Good	94.3	Excellent
	100	62.2	Good	59.3	Fair	79.2	Good
	500	54.8	Fair	51.2	Fair	62.3	Poor
	1000	40.6	Fair	33.4	Poor	52.7	Fair
Cercobin	50	24.5	--	--	--	40.9	Poor
	100	--	--	--	--	17.7	--
	500	--	--	--	--	--	--
Difolatan	50	--	--	--	--	--	--
	100	--	--	--	--	--	--
Ferbam	50	57.6	Good	56.6	Poor	73.2	Fair
	100	55.9	Fair	54.2	Poor	64.6	Fair
	500	45.2	Poor	36.0	--	66.5	Poor
	1000	34.9	--	29.2	--	40.8	--
Plantvax	50	60.8	Good	56.3	Fair	72.0	Good
	100	59.4	Fair	49.2	Poor	54.9	Poor
	500	--	--	--	--	--	--
Thiram	50	66.2	Excellent	56.4	Excellent	68.3	Excellent
	100	54.6	Good	44.3	Fair	62.2	Good
	500	46.0	Poor	22.2	Fair	54.5	Fair
	1000	44.0	--	16.4	Poor	34.3	Poor
Vitavax	50	62.3	Good	53.2	Fair	61.7	Fair
	100	56.2	Good	44.5	Poor	53.4	Poor
	500	46.4	Poor	26.3	Poor	45.4	Poor
	1000	--	--	--	--	--	--
Control	--	82.4	Excellent	78.5	Excellent	68.5	Excellent

EFFECT OF MEDICINAL PLANTS

Effects of some medicinal plant like Neem, Tulsi garlic, onion, madar etc. have been reported to posses chemicals, toxic to various micro-organisms and serve as chemical protective barrier to the infection, antifungal property of several plant extracts has been reported by various workers.

Fungi reproduce and spread mainly by spores. Spores are minute separable bodies with a special form characteristic of the particular species. The commonest type of spore produced by the fungi are asexual.

Germination of fungal spores is essentially a process during which the normal metabolic and physiological activity is restored after dormancy. According to Gottlieb (1964) Germination is the process by which a spore is transformed from a dormant state of low metabolic activity to one of high metabolic activity. Formation of the germ tube is the outward and visible sign that the metabolic change is complete.

He further stated that an alteration should be brought in the conventional concept that spore germination is the process involving the absorption of water with consequent swelling of the spores, causing the cell wall to rupture with the formation of germ tube. These processes are the visible manifestation of a series of complex metabolic changes which take place during the germination of spores. They are dormancy, maturity, longevity, temperature, hydrogen-ion concentration, water and nutrients etc., which inhibit or accelerate the germination of spores. Water is essential to

activate the metabolic activities. The enzymatic activity and the spore germination increases when temperature is near to optimum. Thus the germination of the spores is the result of the action of all the influencing factors operating at the same time.

Spores are known to be more sensitive to environment than mycelium, hence it was considered necessary to investigate the effect of leaf extracts of some medicinal plant on the germination to spores of present fungi at room temperature (25 ± 1°C). Ten day old cultures of the three fusarial pathogens viz. *F. solani, F. oxysporum, F. equiseti* have been used.

Leaves of several plants have been reported to possess chemicals, toxic to various micro-organisms and serve as chemical protective barrier to the infection.

Antifungal property of several plant extracts has been reported by various workers (Sekhawat and Prasad 1971, Mishra et al. 1974 and Mishra, 1975) present work deals with the effect of leaf extracts of a number of medicinal plants on the spore germination of the fungi under study spore suspensions were made in the supernatant extract at two concentrations viz., 100% and 50% and the percentage germination of the spores was recorded after their initial time of germination in the Table - 17.

It is evident from the table that out of five medicinal plant tried leaf extract of Neem (*Azadirachta indica*) at 100% concentration completely checked the spore germination of all the three fungi, while in 100% garlic bulb extract (*Allium sativum*) spores of *F. equiseti* could only germinate up to 20%. Next of Neem and Garlic extracts, Ocimum leaf extract (100%) exhibited antifungal property where spores of *F. solani, F. oxysporum* and *F.*

equiseti could germinate upto 32, 18 and 42% respectively. In comparison to the above leaf extracts spore germination of *F. solani, F. oxysporum and F. equiseti* was 92, 96 and 96 respectively in control sets (distilled water).

Different workers investigated the effect of leaf extracts of various medicinal plants on spore germination of pathogenic fungi. Sekhawat and Prasad (1971) tried leaf extract of *Melia azadirachta Ocimum sanctum* and *Allium sativum* against 41 species of pathogenic fungi out of which *Curvularia penicillium* and *Helminthosporium* spp. were found unable to germinate on *Melia* and *Ocimum* leaf extracts. Mishra et al. (1974) reported complete inhibition of spore germination of *Curvularia. lunata* and *Helminthosporium. graminicola* in leaf extracts of *Melia* and *Ocimum* respectively. Khanna and Chandra (1972) have also reported similar observations for the fungi studied by them.

Table : 17

Showing effect of leaf extracts of various medicinal plants on spore germination of Fusarium species population

S.No.	Leaf extracts	Concentration (ppm)	Percentage of germination		
			F. solani	*F. oxysporum*	*F. equiseti*
1.	*Strychnos nuxvomica L.*	100%	33	24	43
		50%	64	42	81
2.	*Calotropis procera (Ait.) R. Br.*	100%	35	24	47
		50%	57	48	72
3.	*Azadirachta indica A. Juss*	100%	--	--	--
		50%	22	13	42
4.	*Ocimum sanctum L.*	100%	32	18	42
		50%	60	40	62
5.	*Allium sativum L.*	100%	--	--	--
		50%	31	27	63
6.	Control	--	92	96	96

Resistant Varieties

The promise of resistance to pests and diseases has attracted the attention of plant breeders ever since the demonstration near turn of the century that resistance could be selected and that it is often simply inherited. The built in protective resistance effective throughout a crop plant life offers a compelling alternative to fungicides and insecticides. In the early years, minimizing the use of protective chemicals was desirable because it saved money but today there is an added advantage of reducing their hazard against those pests and diseases such as cereal rusts, soil borne smuts, wilt seed borne and certain nematode disease. There is considerable literature available on the breeding and in heritance of disease resistance in plants. A list of papers published on the inheritance of disease resistance in plants upto 1934 has been given by Hanseen (1934).

The literature, on the genetics of disease resistance in vegetables has been reviewed by Walker (1965) and on field crops by Ausemus (1943) and Dickson (1956). Inheritance of resistance to viral disease has been reviewed by Halmes (1954), resistance to rust by Hooker (1967) and to nematodes by Hare (1965). Some more recent review articles are those of Hooker and Saxena (1971), Roane (1972) on trends in breeding for disease resistance in crops, Hooker (1974) on cytoplasmic susceptibility in plant disease and Sadasivan (1975). Alien germplasm as a source of resistance to disease have been discussed by Knott and Dvorak (1976). A recent article is by Browning et al. (1977) on managing hast genes epidemiological and genetic concepts.

The use of disease resistant varieties for controlling plant diseases has been termed as the painless method because it does not cost the farmers anything. The resistant plant defends itself against a potential pathogen by means of a number of physical and chemical characteristics of the plant or which are

formed in the plant in response to infection. The physical characteristics act as mechanical barriers which prevent the entrance and spread of pathogen in plant. The chemical factors, which are toxic to the pathogen, inhibit its growth and activity in the plant.

Since Biffen's (1905) elucidation of the inheritance of the resistance in single Mendelian fashion, spectacular progress has been made in our understanding of the genetic aspects of parasitism and disease resistance. The mechanisms of variability that make the pathogens versatile in their behaviour and host range are now well known.

Flow (1955) explained host parasite interaction in Linseed rust by assuming gene for general relationship between rust reaction in the host and pathogenicity in the parasite.

Hart (1926) observed that the stomata of rust resistant wheat remain closed till late in the morning. By the time they open, the germ tubes of uredospores, formed earlier in the dew, get killed due to evaporation of the water. Link and Walker (1933) reported presence of protocatecuic acid and catechol in the dry Pigmented scales of onion bulbs resistant to *Colletotrihum circinans*. Timonin (1940) reported that resistant varieties of flax excrete hydrocyanic acid (HCN) in the rhizosphere.

Orton (1900) obtained resistant cotton variety from selection and multiplication of resistant individually. He observed that some cotton plants did not show wilting in the heavily infected crop. He collected seeds from these plants and planted them in wilt infested soil. By several such plantings he ultimately got most resistant plants which grew well on heavily infected soils. A vast majority of crop varieties can be attacked by a single pathogen or many different kinds of pathogens can attack a single variety. Most plants are naturally resistant to many

pathogen. During evolution of plant life weak and disease susceptible individuals have been progressively eliminated by nature and the plants which exist today are those having developed resistance to most pathogens in a particular geographic area.

Selection for horizental resistance has been utilized consciously or unconsciously as long as agriculture has existed, but in recent years horizental resistance has become an object in itself for systematic breeding.

In the present study high yielding varieties of *Cicer arietinum* (Gwalior-2, G-24, C-214 Kabuli, Green-seeded B.R. 78) has been tested against seed-borne pathogens.

Pathogenicity tests were performed by rolling surface sterilized seeds on sporulating cultures of the test isolates and planting them in pots filled with sterilized field soil. In another set of experiment healthy seedlings were planted in pots containing infested soil with 3% maize meal inoculum. The experiments were continued for 2 years (1998-99). Healthy seeds of the above varieties harvested in first year of production were sown the next year. The results are summarized in Table - 18.

Result from the experiments clearly show that out of five high yielding varieties of Bengal gram (*Cicer arietinum*) crop tested against *Fusarium oxysporum, Fusarium solani* and *Fusarium equiseti* Kabuli and B.R. 78 (*Green seeded*) was found resistant to the pathogens under investigation.

Table : 18

High Yielding varieties of Bengal gram (*Cicer arietinum*) crop showing percentage infection of three *Fusarium* spp. in two successive year (1998-99)

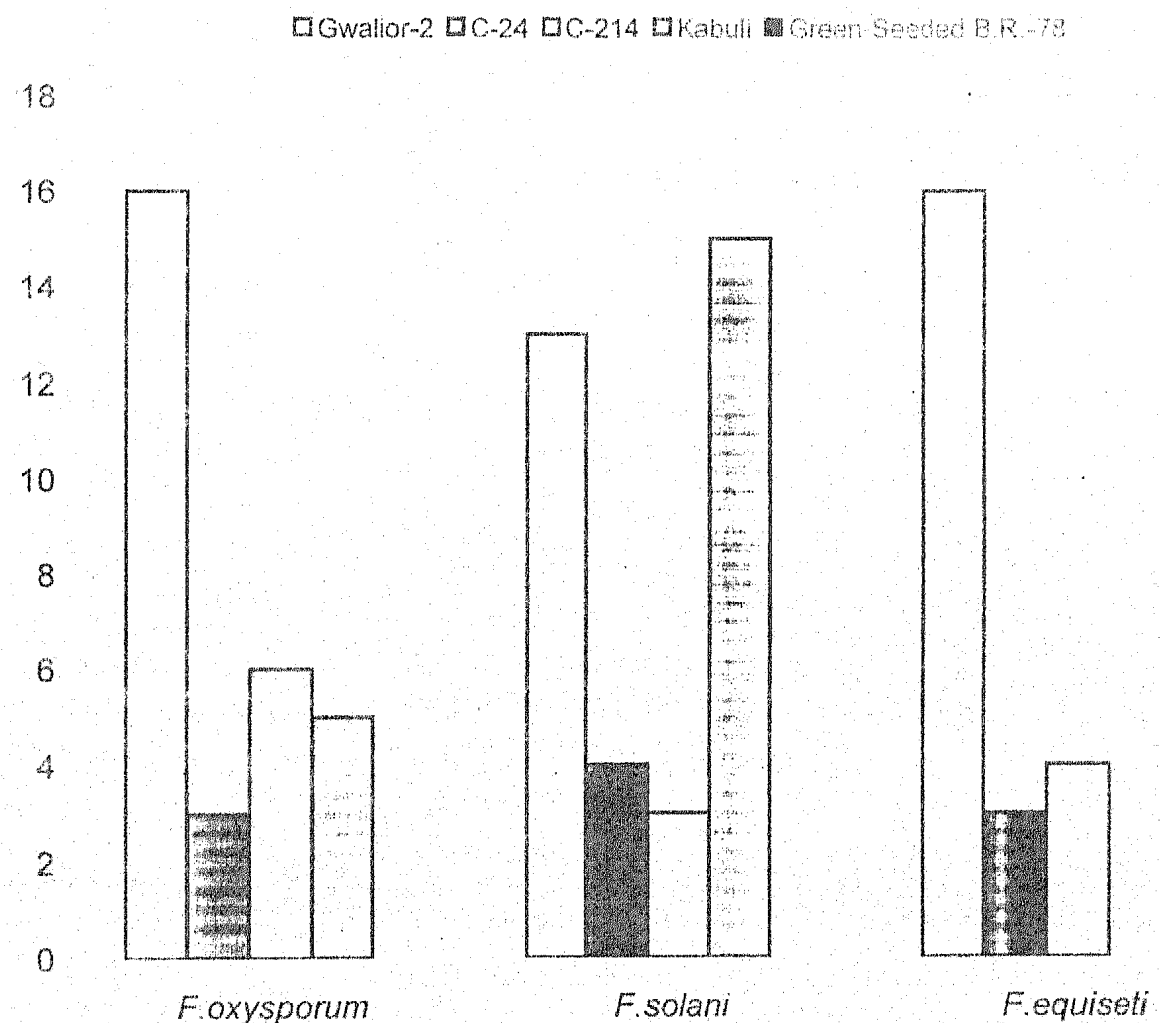
Year - 1998

Varieties	% infected plants		
	F. oxysporum	*F. solani*	*F. equiseti*
Gwalior-2	16	13	16
C-24	03	04	03
C-214	06	03	04
Kabuli	05	15	00
Green-Seeded B.R. - 78	00	00	00

Year - 1999

Varieties	% infected plants		
	F. oxysporum	*F. solani*	*F. equiseti*
Gwalior-2	13	50	14
C-24	04	08	02
C-214	06	03	04
Kabuli	00	00	00
Green-Seeded B.R. - 78	00	00	00

High Yielding varieties of Bengal gram (*Cicer arietinum*) crop showing percentage infection of *Fusarium* spp. in year 1998



CHAPTER - 8

Discussion And Conclusion

DISCUSSION AND CONCLUSIONS

"People need food, not Production Statistics, and a crop is not food until it is eaten. A program to reduce storage losses, probably could result in a 10-20 percent increase in available food now in some of developing countries, and might also assure that whatever increase occur in Production in the future would be used for the nourishment of people, not for feeding Pests".

— Christensen and Kaufman (1969)

Pulses provide essential ingredients to our daily diet and improve the nutritional quality of the Predominately cereal-based diets of large segements of Indian population, Particularly the vegetarians. They contain about two to three times more protein, four times riboflavin and ten times thiamine than that of cereals. Pulses are also relatively rich in two essential micronutrients, viz, calcium and iron than cereals . A Judicious combination of cereal and Pulse protein therefore, considerably improves the overall Protein quality in the diet as a whole. To over come the problem of undernutrition and malnutrition in different Parts of the world particularly in children upto the age of five years, suffering from irrecoverable brain damage leading to intellectual dwarfism which cannot be cured even by intensive corrective measures.

Pulses contain a high protein content which is essential for formation of body tissues. The characteristic fruit in pulses is legume or pod which is formed by the mono-corpellary, unilocular, superior gynoecium with marginal placenation. The Pulses are second only to cereals in their importance as human food, especially in India, when the people derive most of their protein requirements from these crops. Serving as valuable human food, pulse crops are also valued for their Nitrogen fixing quality, in symbiotic relationship with the bacteria Rhizobium.

In recent years, much emphasis has been directed towards increased cultivation of pulses. In the context of multiple cropping, relay cropping, introduction of exotic varieties, application of higher doses of fertilizers, diseases and pests have assumed a special significance's. The technology of plant protection is also undergoing change in concept and methodology.

Pluses are rich in vitamin A, which is three to four times greater when compared with wheat and other cereal. The sporulated grains of pulses contain about 10-15 milligram vitamin C/1000 gm of weight. Pulses are also better source of minerals, especially ca, which is most required mineral by the human system.

Bengal gram is generally consumed by the people as 'dal', 'besan' (Flour), crushed or whole, saltedor Sweet Preparations and green foliage and grain as vegetables. Malic acid and oxalic acid collected from green leaves are a good medicine for intestinal disorders.

At present India is facing acute shortage of pulses crop inspite of large areas of their cultivation. Seed is the basic unit for crop production

and farmer clamour for a few seeds of a new or hybrid variety. However, while introducting seeds and other plant materials from various countries, we have to very careful, so that no serious pathogens are introduced. Due to increase in demand of healthy seeds in developing disease free crop it has become obligatory on the part of phytopathologists, to know a seed pathology". Neergard (1977) has very critically and vividly written a text book on this subject in two volumes. In addition to actual losses due to other hazards, high percentage of losses are recorded as result from seed-borne diseases. These include reduction (i) in actual value of that produce for human use, (ii) in the germination percentage of seeds, which may render seeds unfit for seedling purpose (iii) deterioration of produce in storage and (iv) harmful affects on human or live-stock. Realizing the possible threat from seed-borne diseases, we also need to approach the problem on scientific lines and precise study dealing with important aspects of seed health and seed testing.

Various aspects of seed-borne pathogens have been discussed by Noble (1951, 1957), Noble et al (1958) and By Kestra (1961). Host Parasite relation and environmental influence in seed-borne diseases have discussed by Wallen (1964). Nene and Agarwal (1978) have given information on some seed-borne diseases and their control. Suryanarayan (19787) gives a comprehensive account of the currently important seed-borne diseases. Seed-borne pathogens are of two types :- (i) adherent to the outer covering of the seed, and (ii) borne inside the seed.

Pulses are subjected to many diseases right from the beginning of its life span upto the harvest, caused by fungi.

The studies on isolation of mycoflora from Bengal gram in the present investigations yielded some interesting results. In all twenty fungal genera and single genera of bacteria were recorded from Bengal gram. Seeds, collected from various places, did not show much variation in their fungal flora, although their frequencies were different in each samples.

The fungal isolatets obtained from the above studies were screened for Pathogenicity and among the fungal flora isolated from Bengal gram seeds, for species of *Fusarium* and single species of *Alternaria* was found to be pathogenic on the their respective host. The nature of diseases observed were, foot-root flower-rot, leaf-spot-rot. Seed rot, seedling-blight, stress rot and built. Among the pathogenic *Fusaria, Fusarium oxysporum, F.solani,* and *F equiseti* were observed most frequency to cause a number of disease viz. Seed and seedling rot, will, pod-rot and root-rot.

Investigation on the extra-cellular production of toxins in vitro revealed that *F. oxysporum, F. Salani* and *F. equiseti* were more efficacious in producing the toxins. It was interesting to note that they produced a high amount of *Fusaric* acid in vitro. Thus, The Severity of infection caused by them might be correlated with its capacity to produce high amount of Fusaric acid.

In Leguminosae the seeds after harvest become infected through pods and the infection involved the seed-coat and usually considerable parts of the cotyledone. The site of infection was the fleshy cotyledons, which provided a rich nutritious base for the seedling us well as for the pathogens and possibly accounted for the important role of seed transmission of these pathogens.

Fusarium spp. provided examples of serious seed-borne fungi that penetrated from the vascular system through the funiculus into the seed. Rudolph and Harrison (1945), isolated *Fusarium oxysporum, F. Salani* and *F. equiseti* from vascular bundles in all parts of cotton including the internal tissues of the seed.

Toole (1943) and Harington (1972) mentioned approximate percentage of moisture content in various kinds of seed as related to different degrees of relative humidity of the air. In comparison to seeds having high protein and starch content the moisture content will be lower in seeds having high oil content. The longevity of the seed kept in storage is predominately dependent on the moisture content of the seed and on the temperature and relative humidity in the store room.

Growth and multiplication of fungi depends an moisture level. For each of the common species of storage fungi, there is a minimum moisture content in seeds below which it can not grow. In present problem studies show that, moisture content below 7.5 of all pulse seed, showed absence of any fungal growth. It was interesting to observe that when this moisture level was increased about 7.5% fungal invasion was also increased. At higher percentage (14.5-15.5) heavy invasion by species of *Aspergilli* observed.

Storage studies revealed that there are a number of factors which influence the seed deterioration. In these factors the important factors appear to be moisture content, temperature, the kind of fungal flora involved, the length of storage period storage containers and the rate at which the fungi grow, the condition of seed (Physical and biochemical) and

the number and perhaps the severity of injuries on the seed coat of the seed.

Seed samples stored upto 12 months while studying in storage conditions were heavily associated with both storage and further upto 2.6 years of storage fungal population increased. Slowly. But after 2.6 years of storage it decreased. It was further observed that seeds threshed by machines were more susceptible to fungal flora than bullock threshed seeds may be due to injuries in the former case (Kulick 1973).

Torleson (1947) reported that in humid conditions seeds of Bengal gram are easily damaged by hard threshing, when the moisture content of the seed was high. Flax seeds are also sensitive to threshing injury. The seed-coats are brittle and many easily rupture when the seeds are knocked against a hard surface (Kommendahle et al, 1955)

Many worker reported that dicotyledonous seeds are particularly sensitive to thresher injury, which is beans may produce upto 30 percent abnormal seedlings (Harter 1930).

Isolation from seeds collected from various containers showed that there are lesser fungi in the containers and sacks kept in wheat straw than seeds stored in earthen pots and on floor.

Dungan and Kochler (1944) recorded that, in maize, *F. gramineaum* dried out completely in one year. Most of the seeds being free of the pathogen already after two years. On the other hand, some of the seeds still harboured viable *F. moniliforme* after eight years. *F. oxysporum* in seed of red clover could still be isolated after six years of storage in air-tight

containers at 5 percent relative humidity of the air (Narkiewicz-jodko, 1974).

Shands (1937) found that *Fusarium graminearum* could live in barley upto 27 months, Christensen (1963) recorded in 20 months, *F. culmorum* and *F. avenaecum* were viable even a shorter period of time Shands (1937) whereas Ponchet (1966) recorded survival of *F. nivale* although in low percentage in wheat after 42 months.

Many species of *Alternaria* commonly encountered in seed are long lived under storage of the seed usually atleast 5-6 years (Neergard 1945). In barley A tenuis viable after storage for 6 years (Christensen, 1963). Lutey and Christensen (1963) found substantial reduction in percentage of field fungi such as *Alternaria*, *Drechsera* and Particularly *Fusarium* in barley kernels kept for a feed months at 14 percent moisture content as 20°C.

Kerr (1963) reported root-rot and *Fusarium* wilt complex of pea to be caused by *F. Salani. F. sp. pisi. Fusarium oxysprum* is reported by various workers as wilt causing pathogen of many hosts and most of these are seed borne (Noble and Richardson, 1968 and Anderson (1974). It may cause stem-rot and foot-rot of the plants. *F. moniliforme* is known as casual agent of seedling blight,, foot-rot stunting and hypertrophy (Both 1971) and as seed-borne (Nobel and Richardson, 1968, Ram Nath et al., 1970 and Anderson 1974).

Fusarium solani causing wilt in *Psidium guajava, Abelmoschus esculentus* and *Cicer arietinum* respectively reported by chattapodhya and

Sengupta (1955) Chattopadhya and Basu (1957).

Benoit and Mathur (1970) have monographed seed-borne species of *Curvularia* and described their habit on seed and reviewed their occurrence as pathogens mainly on cereals. Mathur et al (1973) reported *Chaetomium globosum* on seeds of *Pennisetum typhoides.*

Alternaria alternata was responsible for causing the leaf-spot diseases of their respective hosts at different stages of plant growth. *Alternaria alternata* is reported by Groves and skolke (1944) and Neergard (1945) on different seeds as saprophyte.

Macrophomina phaseolina is reported as causal agent of charcol-rot, stem-rot, ashy stem-blight (Noble and Richardson, 1968) Watanable (1972) and Chidambarum and Mathur (1975). *Rhizopus arrhizus* and *R. stolonifer* have been reported by Venkatram (1950) and Halisky and Satour (1964) as part of ball-rot organism of cotton, leading to internal infection of seed. Trichothecium roseum is a common Saprophyte on Seeds (Doyer 1938 and Malane and Masketl 1964). Many physical, biochemical and physiological deterioration in seed is caused by microbial infestation after harvest. Poor and improper storage conditions increase the rate of deterioration. The micro-organisms growing on seeds secrete metabolites which cause biochemical degradation of seed substances. There are many complex seed constituents which are affected due to mouldiness of the seeds.

In the present problem, studies were done on changes in protein of Bengal gram seeds. The results shows that within the incubation period upto 45 days) the fungi viz, *F. salani* and *Aspergillus flavus* were not able to

cause any markable loss of protein than *Fusariam solani*. Inter-relationship studies between seeds and seed-borne fungal flora revealed that fungal spore germination in seed-coat lechates and seed extracts decreased, in comparison to contrast sets. In seed-coat leachates the inhibition was more pronounced. It may be due to presence of some antifungal substances in seed-coats. It was also noticed that culture filtrates of selected organisms checked that seed germination and seedling growth remarkably.

Seed mycoflora plays an important role in bringing about the disease development in field. Therefore it is essential to find out possible control measures for these maladies.

The primary screening of eighteen fungicides was determinet against three *Fusarium* species, among them four were found to be effective, as they chcek the mycobial growth. The efficacy of effected Fungicides was tested against growth of fungi on seeds, and to control diseases coming on seedlings and plants. It was observed that the fungal population was reduced with the fungicidal seed treatment and there is no sign of harmful effect on seed germination too.

Dharmvir and Grewal (1961) and Dharmvir et al (1970) have established the superiority of some of these fungicides in control of some seed-borne diseases, were infection is deep seated. Seed-borne infection of *F. Semitectum* was controlled by Seed-treatment (Saharan and Gupta, 1974).

Nene et al. (1969), Nene (1971), Nene and Srivastava (1971), have also shown that fungicide seed treatment have improved seed emergence.

On the basis of experimental work conclusion comes out that proper care of Bengal gram seeds should be taken to prevent fungal deterioration. Necessary care should be taken to avoid injury to the seeds. Proper storage of seeds should be done after-dried well it. They should be stored either in tin containers in gunny bags (Sacks) covered with straw, for consumption purposes.

Fungicidal treatment should be given *Cicer arietinum* to save the germ and check the development of various diseases caused by *Fusarium oxysporum, F. Solani* and *F. equiseti* the Fungicides are Benlate, Cercobin, Difolatan, Plantvax and Vitavax at 50ppm, 500 ppm, 100 ppm, 1000 ppm. Difolatan was found to be more effective.

In the case of *Ustilago nuda* and *Ustilago tritici* recent researches have been done on floral infection in Field and pathway of infection of the seed. Penetration usually takes place through the ovary wall and not via the stigma and style as was earlier proposed (Batts, 1955) The hyphae cross the cells of pericarp (fruit-coat), enter the testa (Seed-coat) and then move towards the ovary. After reaching the ovary the hyphae turn sharply from the testa, enter the scutellum and pass into the growing point of the embryo, mycelium is intracellular, within the pericarp and testa but it becomes intercellular within the tissues of the embryo.

There is no doubt about the role of temperature on the occurrence and development of diseases. Blight of wheat is caused at a comparatively higher temperature that is 20-28°C. In the case of maize, several diseases is caused when the soil temperature is between 16°C and 20°C.

Temperature is an important environmental factor affecting the metabolic activities of the fungi. It is quite clear from the observation that growth, sporulation and chlamydospores formation of the organism *F. oxysporum, F. solani* and *F. equiseti* were prominently influenced by temperature variation. Both species could grow between a range of 10°C and 30°C. Their were recorded at 20°C.

No chlamydospors were found at 20°C except in case of *F. solani* Chlamydospores formation were better in both *Fusarium* species when the temperature was below and above 20°C.

Atmospheric humidity and precipitation in the form of rain, fog, dew, etc. determine disease incidence to a great extent. Like wise, the amount of soil moisture determines the severity of disease in soil. The speed of germination of inoculum, its entry into the host and the period of incubation are very much affected by the amount of moisture available.

Further, a novel idea of controlling seed-borne diseases by leaf extracts revealed that leaf extracts of *Azadirachta indica, Ocimum sanctum* and *Strychnos nux-vomica* controlled fungal seed contamination. Leaf extracts of the medicinal plants checked the spore germination of the fungi investigated. The possibility of the presence of different chemical compounds, fungicidal or fungistatic in action in these extracts which exerted inhibitory influence upon germination of fungal spores was always there.

While studying effects of leaf extract of medicinal plant on spore germination and disease development, leaf extract of five medicinal plants which were found effective on spore germination of *Fusarium* spp. Neem

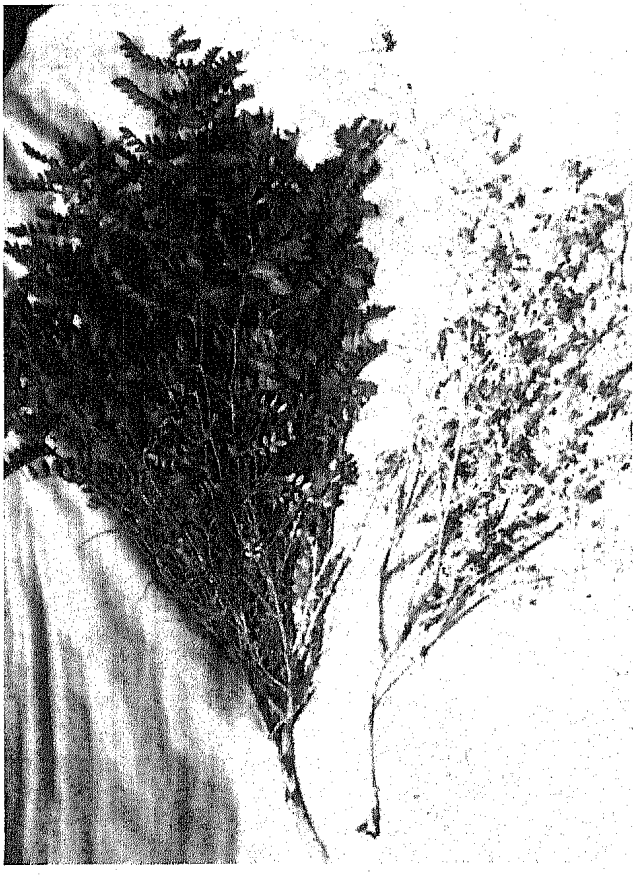
(*Azadirachta indica*) at 100% concentration, completely checked the spore germination of both *Fusarium* spp. It was further observed that *Allium sativum* leaf extract at 100% concentration was also proved effective to check the spore germination of test organisms. Third effective leaf extract was *Ocimum sanctum* studies on high yielding varieties revealed that out of yielding varieties of Bengal gram tested, two (kabulee and BR 78) were found to be resistant to all the pathogens most of the field Fungi were sensitive to high temperatures and usually disappeared under such temperature conditions (Lutey and Christensan 1963). *Alternaria alternata* and *Rhizopus stolinifer*, however, developed under temperature above 40°C (Helberg and Kolk 1972).

Biological control has been broadly defined as the encouragement of beneficial organism already existing in locality or of the introduction of suitable new species of exotic parasitic organisms, which are parasites or harmful pathogens in a locality where the pest in thriving with a view of control the disease. Though the practical application of biological control is of comparatively a recent origin, interactions between different organisms in an ecosystems must have been existent for the maintenance of a stable population in a environment.

The effect of 15 days of fungal culture filtrates on seed infestation showed that percentage seed contamination was significantly low as compared to control sets. Seed-coat leachates were also found to inhibit to the fungal spore germination and decreased the fungal seed contamination. This was probably due to presence of some antifungal substances.

CHAPTER - 9

Summary



SUMMARY

Bengal gram (*Cicer arietinum*) is the most important pulse accounting for more than one third of the area and about 40 percent of the production in the country. Dal, besan (flour), crushed or whole gram, boiled or parched, roasted or cooked, salted or unsalted or sweet preparations and green foliage and grain, as vegetables, are the important form in which it is consumed by the people. Germinated seeds are recommended to cure scurvy. Malic and oxalic acids collected from green leaves are prescribed for intestinal disorders.

The average annual area and production of Bengal gram are about 7-8 million hectares and about 4-5 million tonnes respectively. Northern India accounts for nearly 90 percent of the annual area and about 95 percent of the production, Uttar Pradesh, Himanchal Pradesh, Rajasthan and Haryana accountion for more than 6 million hectares.

A comprehensive survey of various markets of Allahabad, Banda, Mahoba, Hamirpur Mauranipur (Jhansi), Agricultural Testing and Demonstration centre Lahartara (Varanasi), I.I. P.R. Kalyanpur (Kanpur) and its adjacent regions was made and various seed samples of 'Bengal gram or channa' crop was collected. Blotter and the Agar plate methods were use for detection of seed borne and surface fungi. An attempt was also made to isolate fungi from some split pulses.

Various samples of seeds of Bengal gram crop collected from I.A.R.I., New Delhi, Allahabad Agricultural Institute, Allahabad, G.B. Pant

University of Agriculture and Technology, Pant nagar and from local markets. The fungal culture were purified and maintained on Malt Extract and Potato Dextrose Agar media, for further examination and pathogenicity tests. Morphologiacal studies were carried out and identifications were made.

Fungi isolated from Bengal gram (*Cicer arietinum*) : *Fusarium solani (Mart) Sacc., Aspergillus niger, Pestalotia spp., Phoma putaminum, Penicillium spp., Fusarium oxysporum, Curvularia lunata, Fusarium spp., Aspergillus flavus, Botryodiploidia theobromae, Phoma emblica, Aspergillus spp., Rhizopus stolonifer, Fusarium semitectum, Macrophomina phaseolina, Fusarium equiseti, Fusarium moniliforme Sheld.*

The isolation studies revealed that mostly species of *Fusarium* were associated with the disease syndrome. In a few cases *Macrophomina phaseolina* and species of *Rhizoctonia* were also observed. Apart from these species of *Curvularia lunata, Rhizopus spp., Penicillium, Aspergillus niger* were also isolated from the rhizosphere and rhizosplane of the diseased roots.

On root *Macrophomina phaseolina* was dominated in summer while *F. solani, F. oxysporum* and *F. equiseti* were dominant in winter season. A close relationship was observed in number and types of fungi isolated in all the above cases.

Free hand and microtome section of the diseased roots showed blocked xylem vessels and *F. oxysporum* and *F. solani* were isolated from rootlets.

Pathogenicity test were conducted simultaneously. For this purpose *Fusarium* species were grown on 3% maize-meal medium and the fungal inoculum was obtained in sufficient quantity which was further incorporated in the soil of the post containing seedlings. The plant of two age groups one week old seedings and 8 weeks old plants were selected or preliminary studies. The results of pathogenicity tests have been recorded as follows.

Pathogen	**Disease**
Alternaria alternata	Leaf-spot
Fusarium solani (Mart) Sacc	seed-rot, seedling-rot and Wilt
Fusarium moniliforme	Wilt and seeding-rot
Fusarium equiseti	Root-rot, wilt and seed-rot
Fuasrium oxysporum	Wilt, seed-rot and seeding-rot
Fusarium acuminatum	Root-rot and wilt

Plant pathogenic fungi not only derive nourishment from the host but various toxic metaboliters produced by them after various wilt diseases is well known. In the present investigation detection of Fusaric acid was done in *F. solani, F. oxysporum* and *F. equiseti.*

Ecological studies showed that much variations in the *Fusarium* population were observed round the three years, but the trend of *Fusarium* population in the soils of twelve different area was similar. In all the cases maximum number of colonies were isolated in October. Minimum number of colonies were isolated during July.

The present study dealing with the effect of some factors on the

survival of species of *Fusarium* in soil induced that lowering or raising the temperature of the soil or by increasing the soil moisture, the wilt of Bengal gram may be controlled to some extent. The population of *Fusarium* was considerably reduced at low or high temerature and high soil moisture amendments of soil with certain carbon sources also decreased the *Fusarium* population. It was noted that an increase in C:N ratio of soil directly proportional to the decline of *Fusarium* population.

Experiments dealing with effect of fungicides in vitro indicate that the effect of Benlate and Difolatan were inhibitory at lower concentrations to all the present species of *Fusarium.* difolatan at 100 ppm could almost completely eliminate the *Fusarium* population from the soil.

The leaf extracts of five medicinal plants were used to check the fungal contamination. Leaf extracts of *Strychnos nux-vomica, Calotropis procera, Azadirachta indica, Ocimum sanctum, Allium sativum* at 100% and 50% concentration tried, in which *Azadirachta indica* and *Allium sativum* at 100% concentration, completely checked the spore germination of the three fungal sp. viz., *Fusarium oxysoproum, Fusarium solani* and *Fusarium equeseti.*

Studies on high yielding varities to seed-borne fungi viz : Fusarium oxyspurum, F. solani and F. equiseti revealed that out of six varities of Bengal gram tested, two Kabuli & BR - 78 were founded to be resistant.

Effect of culture filtrates of fungi on fungal seed infestation has also been observed. Seed-coat leachates also decreased the spore germination and fungal contaminations. Bengal gram tested, reduced the fungal contamination up to some extent.

Market demand for the ulternative to chemical fungicides is bound to incresed in the country and in world in future. That is because Indian goverment has recently announced ban of nearly 300 chemical products. The significance of present study deserves to be assessed in light of environmental awakening all around the world. It the process of sustainable devlopment which will determind the future destiny of mankind.

CHAPTER – 10

References

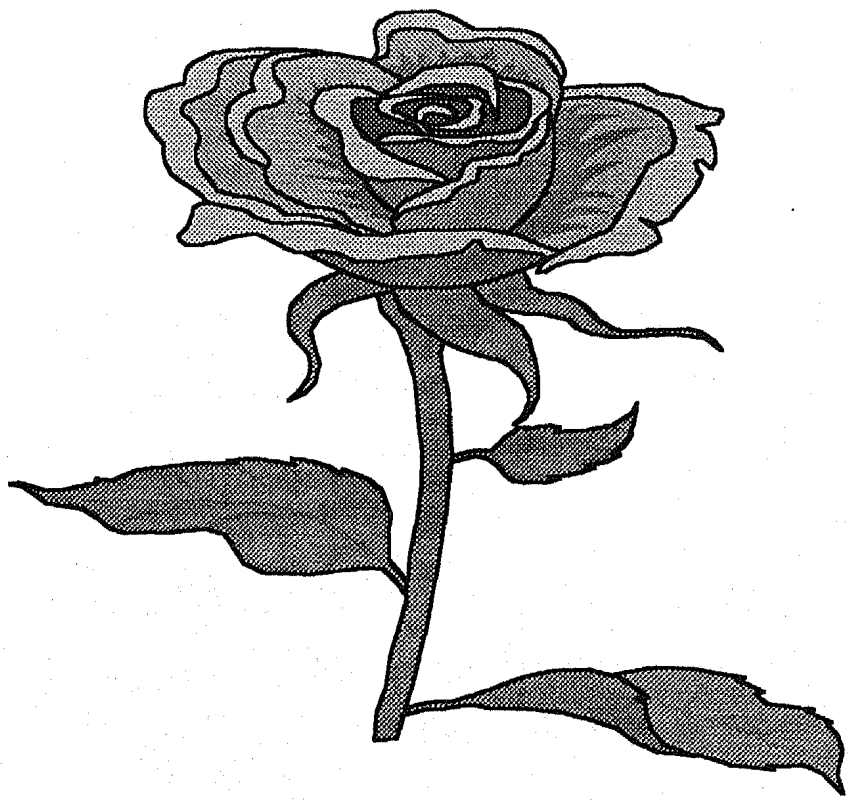


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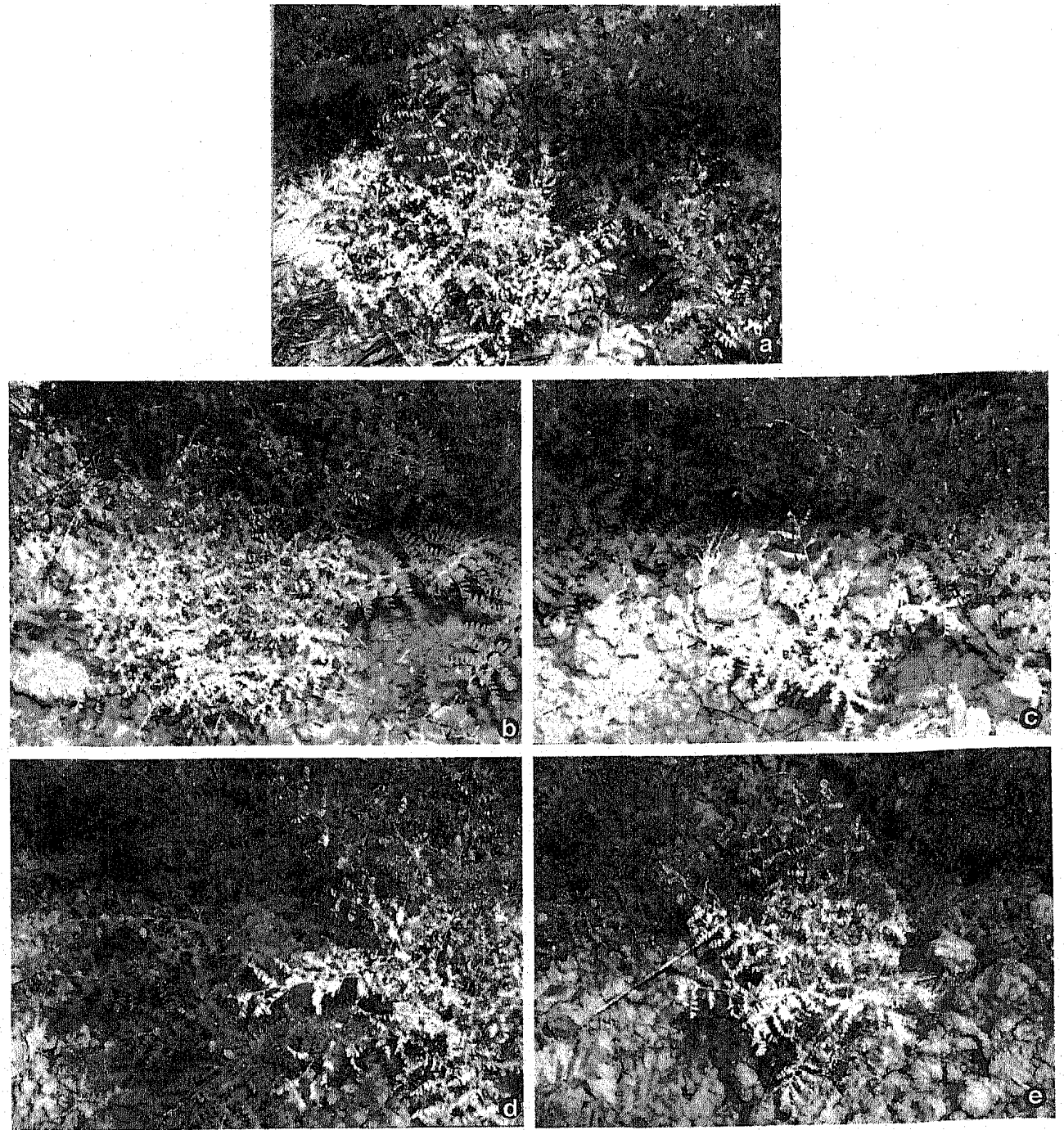
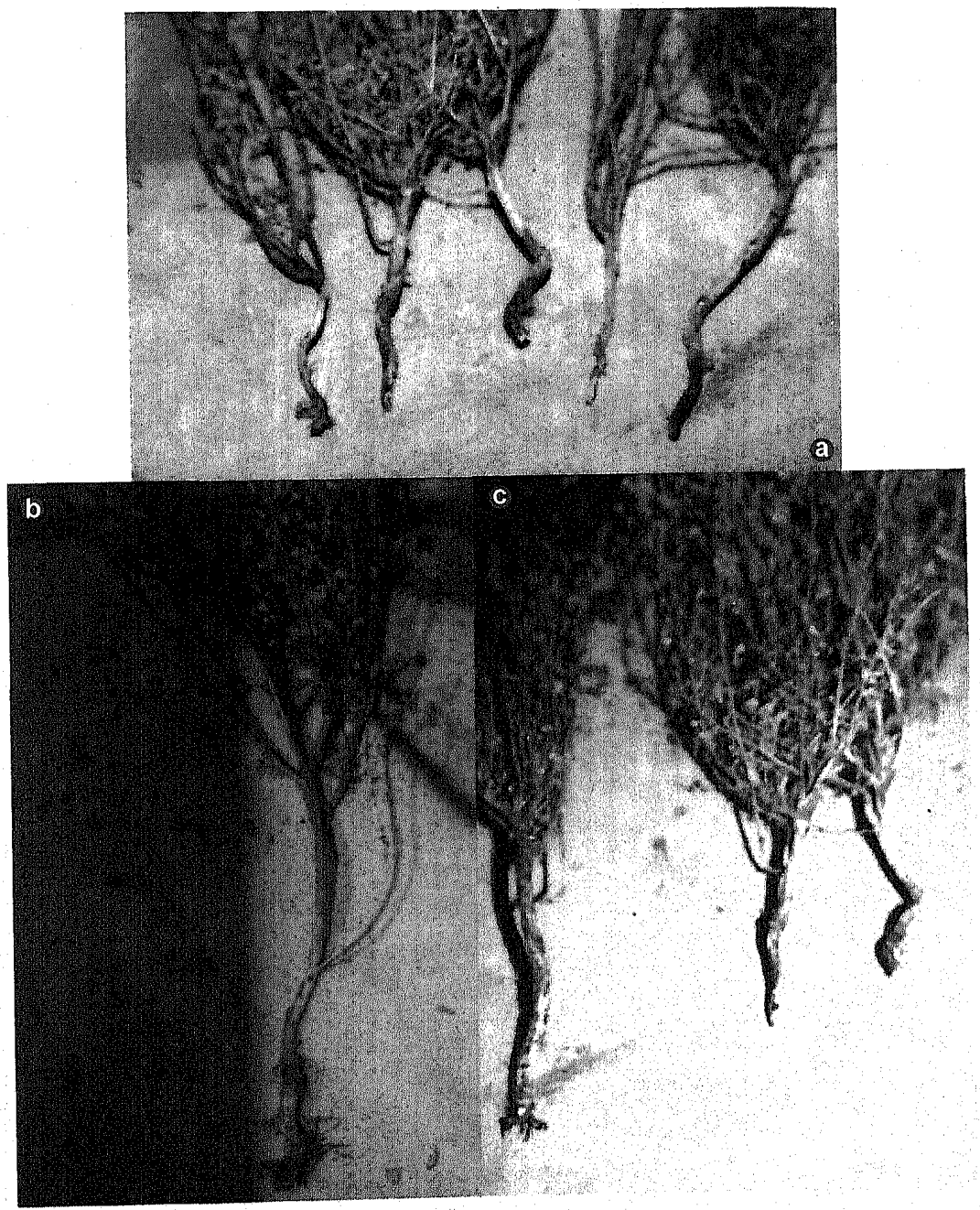
PLATE - 1



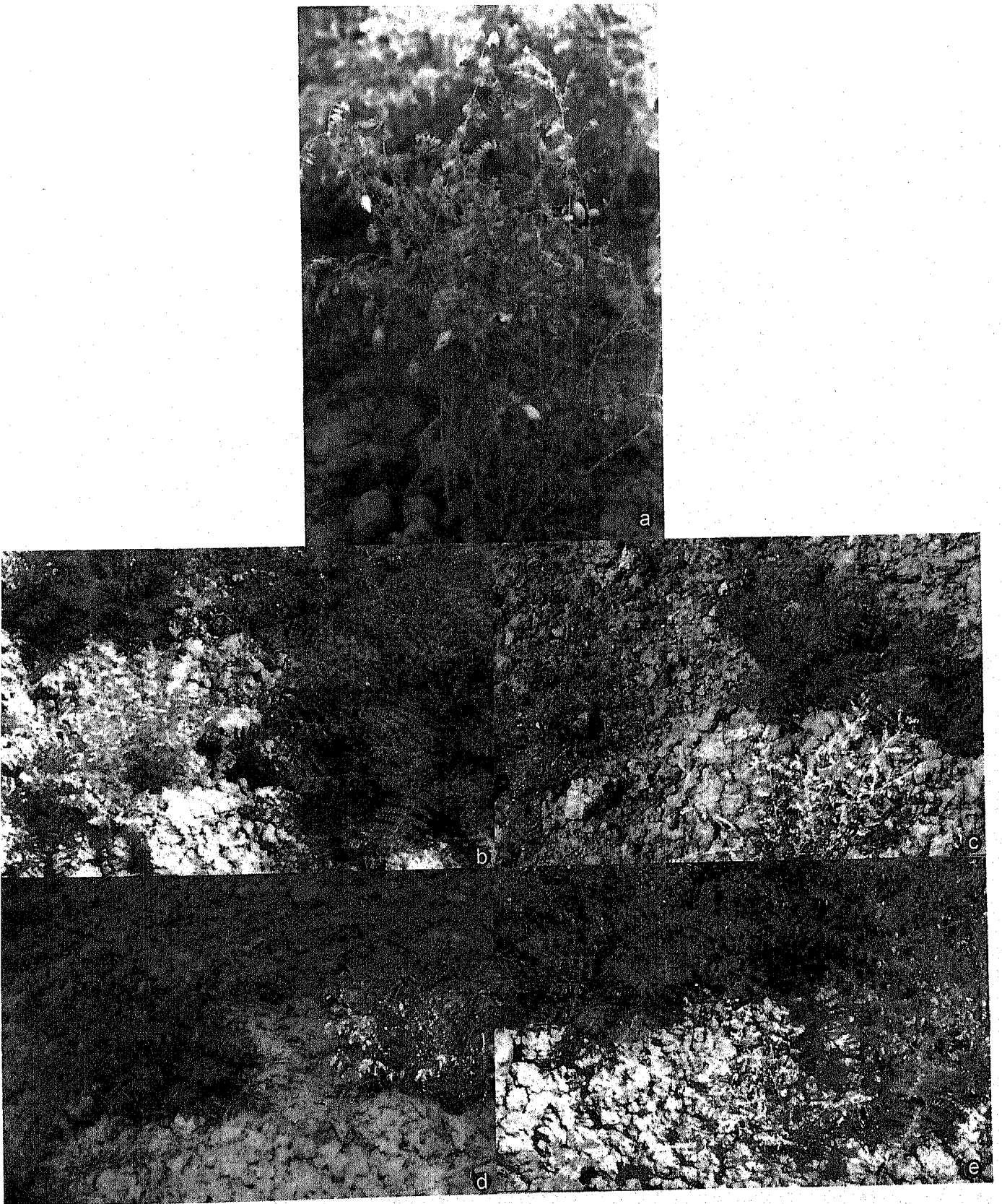
Fusarial (*Fusarium oxysporum*) wilt of Bengal gram (a,b,c,d,e)

PLATE - 2



Fusarial (*Fusarium equiseti*) Root rot of Bengal gram (a,b,c)

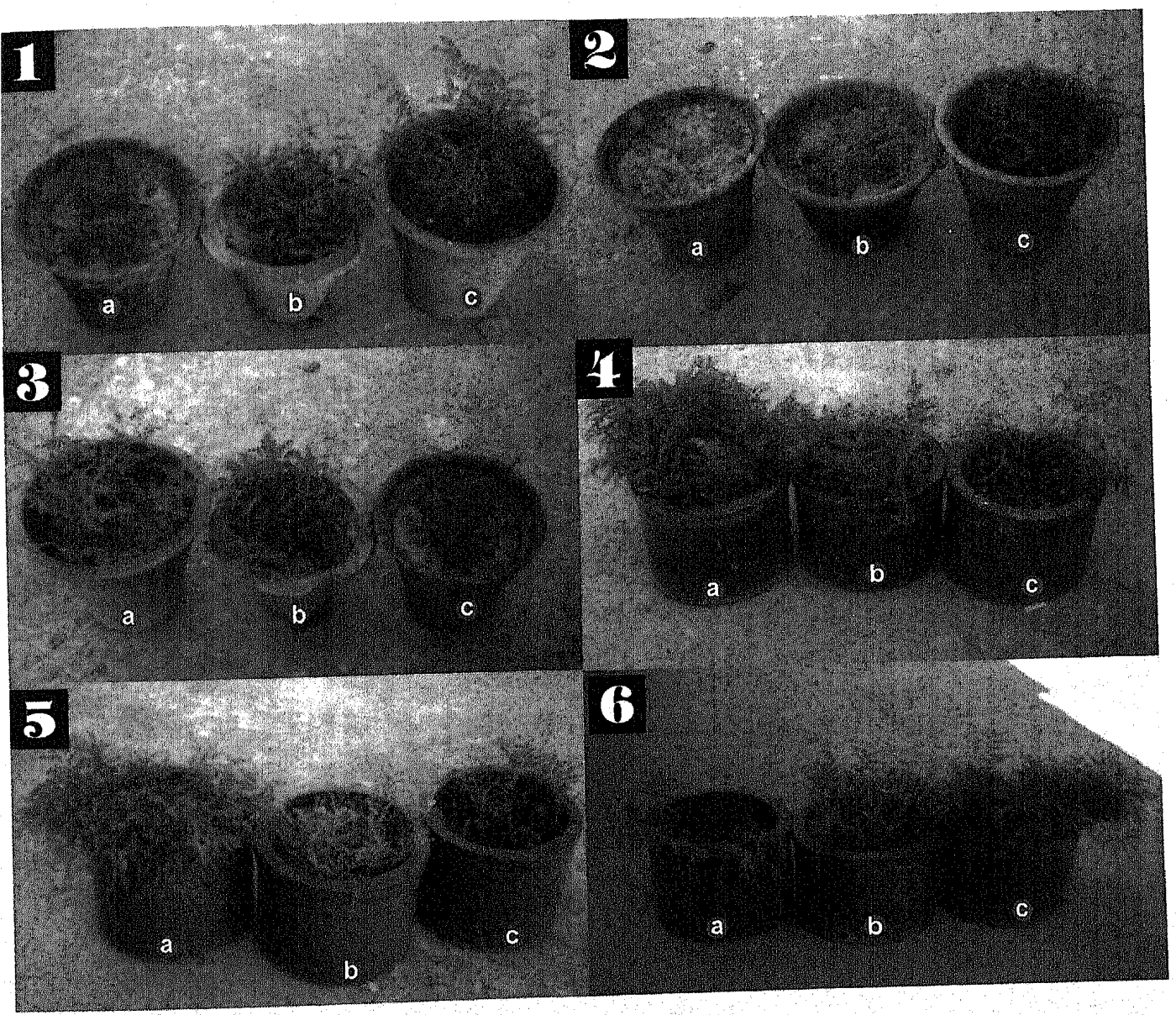
PLATE - 3



Fusarial (*Fusarium solani*) Wilt of Bengal gram (a,b,c,d,e)

PLATE - 4

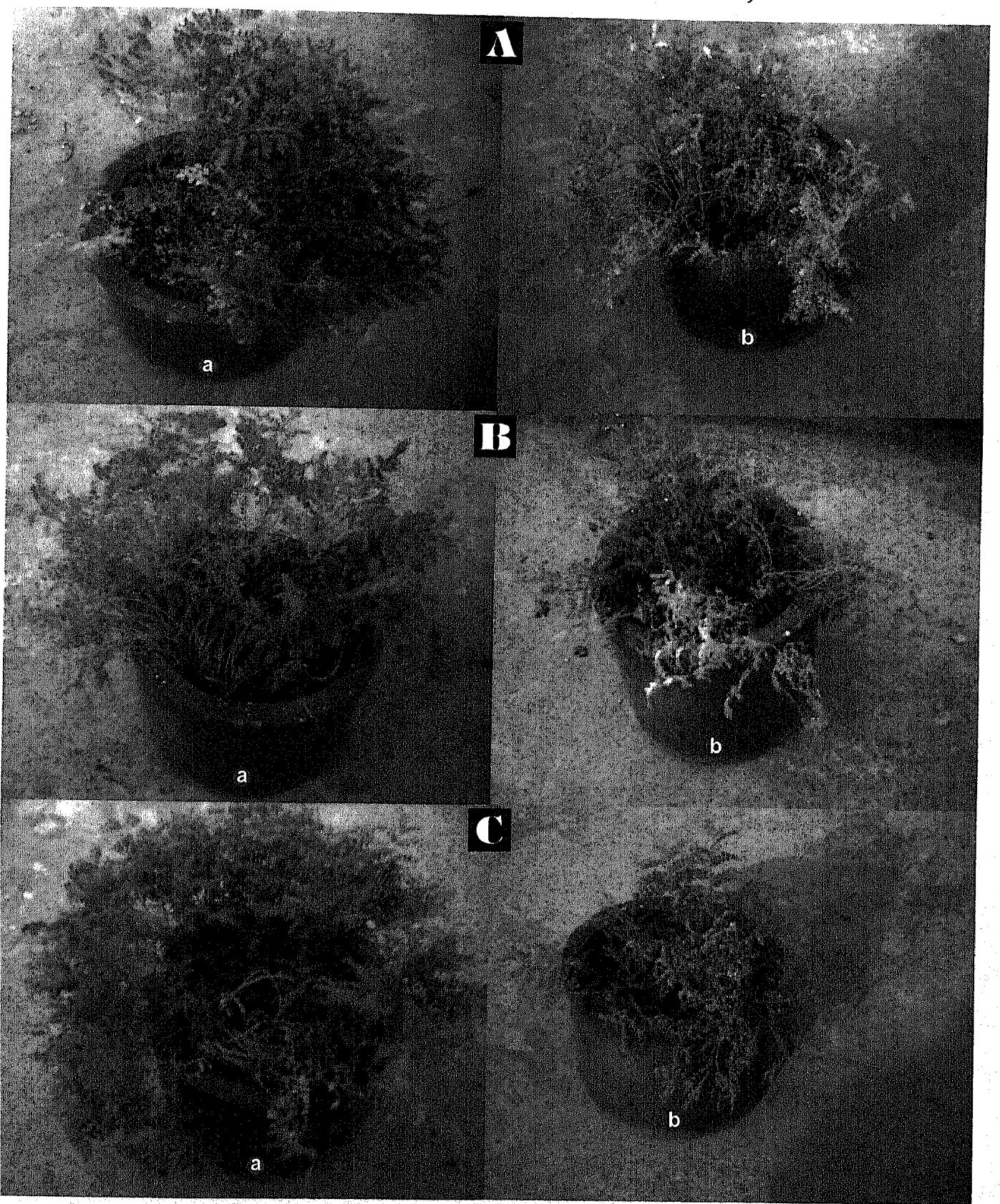
Fusarial wilt of Bengal gram (*Cicer arietinum*)



1- (a) *F. oxysporum* (b) *F. Solani* (c) Control
2- (a) *F. oxysporum* (b) *F. Solani* (c) Control
3- (a) Control (b) *F. Solani* (c) *F. equseti*
4- (a) Control (b) *F. oxysporum* (c) *F. equseti*
5- (a) Control (b) *F. Solani* (c) *F. equseti*
6- (a) *F. oxysporum* (b) *F. equseti* (c) Control

PLATE - 5

Fusarial wilt of Bengal gram (*Cicer arietinum*)



A. (a) Control, (b) Infected (*F. oxysporum*)

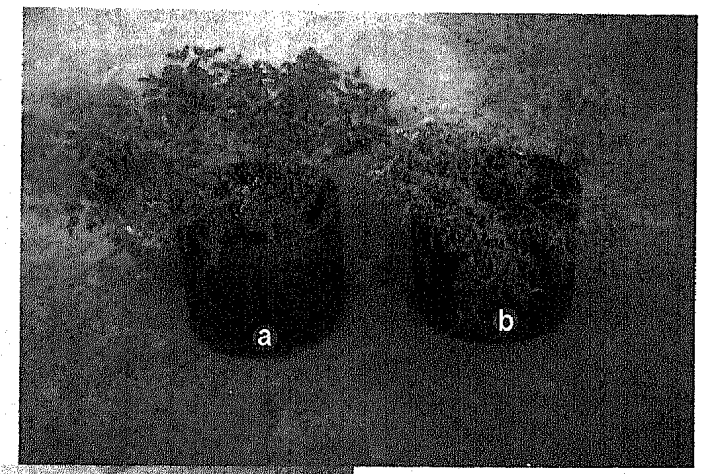
B. (a) Control, (b) Infected (*F. solani*)

C. (a) Control, (b) Infected (*F. equseti*)

PLATE - 6

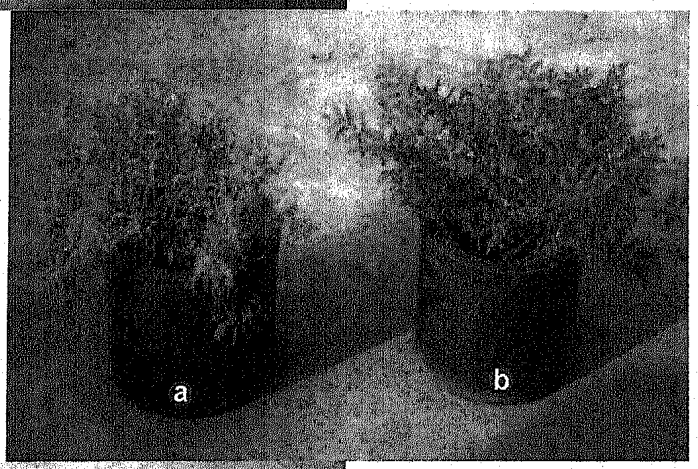
Fusarial wilt of Bengal gram (*Cicer arietinum*)

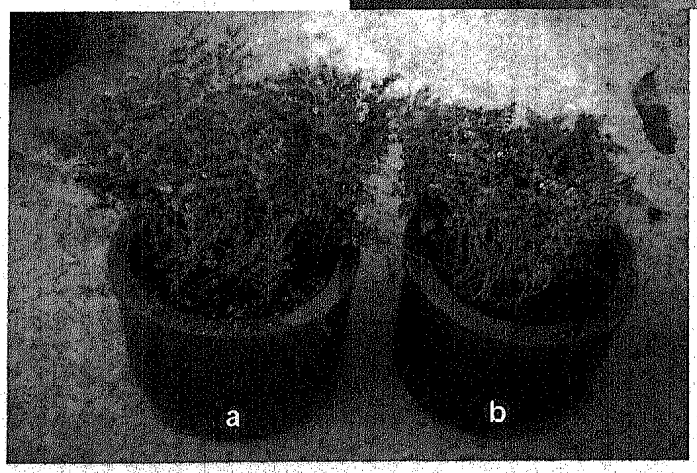
(a) Control,
(b) Infected (*F. oxysporum*).



(a) Infected (*F. solani*),
(b) Control.

(a) Infected (*F. equiseti*),
(b) Control.



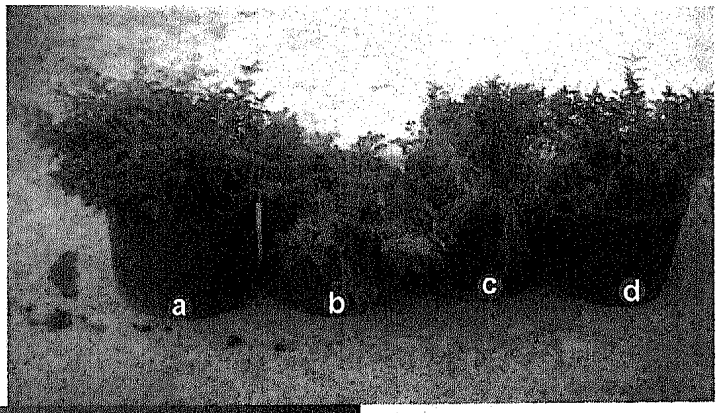


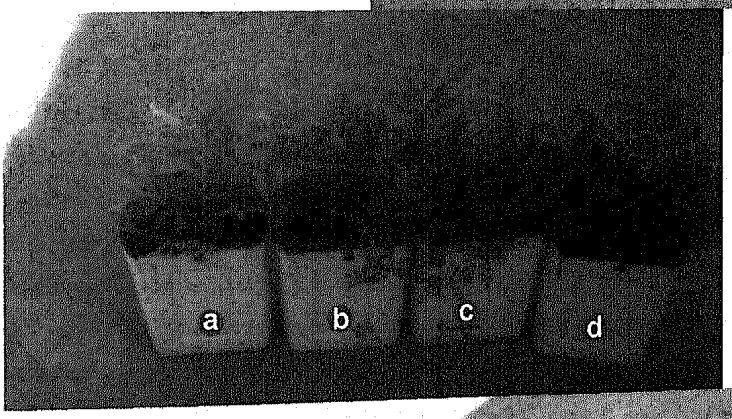
(a) Infected (*Fusarium* species),
(b) Control.

PLATE - 7

Fusarial wilt of Bengal gram (*Cicer arietinum*)

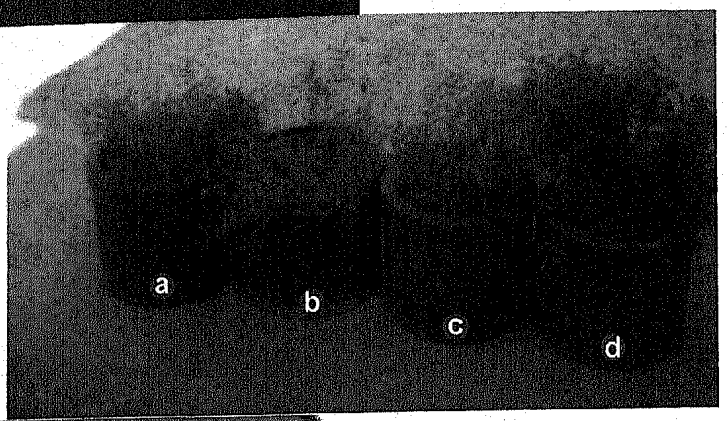
(a) Control,
(b) *Fusarium oxysporum*,
(c) *Fusarium solani*,
(d) *Fusarium equiseti*.

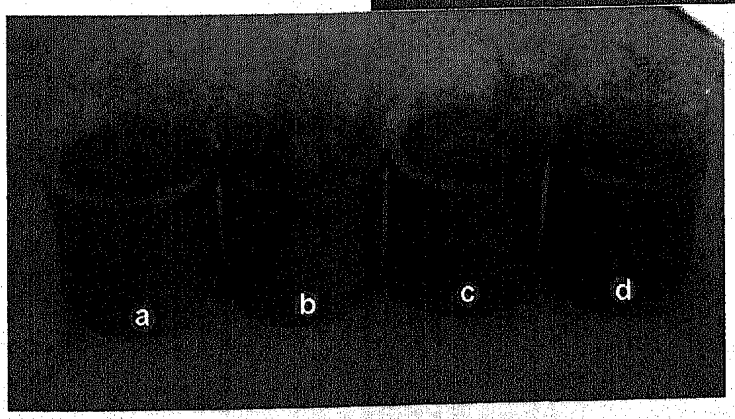




(a) *Fusarium oxysporum*,
(b) *Fusarium solani*,
(c) *Fusarium equiseti*,
(d) Control.

(a) *Fusarium oxysporum*,
(b) *Fusarium solani*,
(c) *Fusarium equiseti*,
(d) Control.

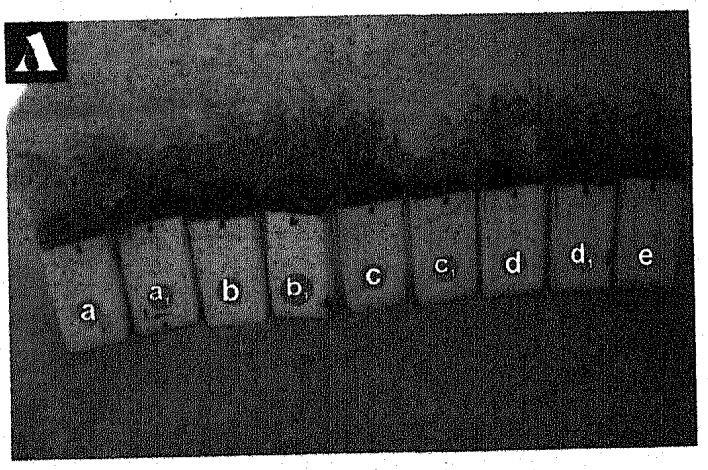


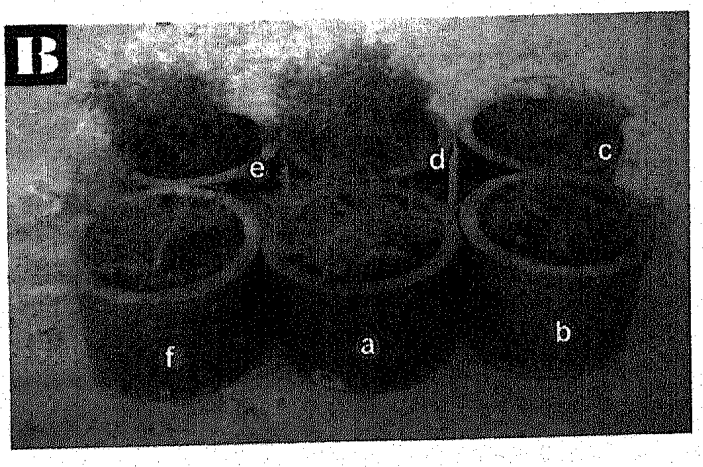


(b) *Fusarium oxysporum*,
(c) *Fusarium solani*,
(d) *Fusarium equiseti*,
(c) Control.

PLATE - 8

Wilt resistant varieties of Bengal gram (*Cicer arietinum*)





A - (a&a_1) : Kabuli, (b&b_1) : BR-78, (c&c_1) : Gwaliar-2, (d&d_1) : C-24, (e) C-214

B - (a) Kabuli, (b) Gwaliar-2, (c) C-24, (d) BR-78 and (e) C-214